

## INTRODUCTION

The discovery made by Osborne and Mendel (1915) that growth in animals could be resumed after it had ceased due to deprivation of food touched on an area of growth physiology that was previously unexplored. Subsequent research has shown that the ability to resume growth without stunting is a naturally occurring phenomenon in many wild fish stocks (Gaylord & Gatlin, 2000). More recently, investigations into compensatory growth in fishes have sought to gain a better understanding of this process.

Compensatory growth is the period of unusually rapid growth which occurs after an episode of under-nutrition. Typically, compensatory growth is accomplished through an increase in appetite (hyperphagia), and/or increased feeding efficiency (Miglav & Jobling, 1989; Quinton & Blake, 1990; Hayward *et al.*, 1997; Gaylord & Gatlin, 2000; Xie *et al.*, 2001). A lack of food resources or decreased temperatures (Nicieza & Metcalfe, 1997) can cause fish to employ compensatory growth as a means to return to their optimal growth trajectory following a period of poor growth (Xie *et al.*, 2001).

Numerous studies have been conducted to observe the compensatory growth response of different species, both marine and freshwater, using a variety of different methods. Full compensation has been documented in rainbow trout, *Salmo gairdneri* (Dobson & Holmes, 1984; Quinton & Blake, 1990), Channel catfish, *Ictalurus punctatus* (Gaylord & Gatlin, 2000), and Atlantic salmon, *Salmo salar* (Nicieza & Metcalfe, 1997). Partial compensation has been observed in Alaskan yellowfin sole, *Pleuronectes asper* (Paul *et al.*, 1995), Arctic charr, *Salvelinus alpinus* (Miglav & Jobling, 1989; Jobling *et al.*, 1993), Atlantic silversides, *Menidia menidia* (Schultz *et al.*, 2002), and in cyprinids (Wieser *et al.*, 1992). Most notable, however, was the study by Hayward *et al.* (1997)

which successfully doubled the growth rate of hybrid sunfish through cyclical manipulations of their feeding regime. (For a comprehensive review of all compensatory growth literature, see Ali *et al.*, 2003). By alternating feeding and starvation periods, the compensatory growth response was successfully elicited, and the increased growth rate was maintained over the duration (105 days) of the experiment. Especially important to Hayward's study was the strategic use of hyperphagia to gauge the duration of re-feeding periods in order to maximize and extend the compensatory growth response. Although Hayward *et al.* achieved over-compensation in their study, it remains unclear whether this is a phenomenon unique to hybrid sunfish or has applications to other species. Unfortunately, interspecific variation in the capacity for compensatory growth is poorly understood. It is not clear whether the apparent differences reported by studies thus far are due to a species effect or whether they are due to the different methodologies used to evaluate compensatory responses (Gaylord & Gatlin, 2000; Xie *et al.*, 2001; Zhu *et al.*, 2001).

Aquaculture was first practiced by Asian cultures over 2000 years ago (Diana, 1995), and initiated the pursuit to understand factors controlling fish production. Today, with a human population undergoing exponential growth and shrinking agricultural areas due to increased development, it is more important than ever to attain food resources that can meet global demands. The science of aquaculture is expanding rapidly to fulfill this need, and important goals of modern aquaculture are to maximize fish production and the efficiency of production. Thus, when choosing a subject species for aquaculture, certain fish are valued over others due to the suite of traits they possess. Both black sea bass (*Centropristis striata*) and southern flounder (*Paralichthys lethostigma*) have been

identified as promising candidates for aquaculture (Berlinsky *et al.*, 2000; Benetti *et al.*, 2001) based on their desirable characteristics.

The black sea bass (*Centropristis striata*) is a member of the family Serranidae (true sea basses) whose range spans the U.S. Atlantic and Gulf of Mexico coasts (Musick and Mercer, 1977). There are two recognized stocks for this species in the western Atlantic, a northern stock and a southern stock, divided at Cape Hatteras, North Carolina (Shepherd, 2000). Additionally, the Atlantic and the Gulf of Mexico populations have been identified as separate subspecies, *Centropristis striata striata* and *Centropristis striata melana*, respectively (Bartone, 1977). Black sea bass (BSB) are protogynous hermaphrodites, living their first 2-5 years as females, then transforming to males (Shepherd, 2000). Spawning is initiated in the shelf waters (20-50 m depth) off of North Carolina in March, and progresses northward through October (Shepherd, 2000). Juveniles spawned on the shelf move inshore into estuaries, bays and sounds where they shelter in beds of aquatic vegetation, oyster reefs, wharves, pilings, and other structures, and then migrate offshore in December. These temperate reef fish may live as long as 20 years, although females are rarely found older than 8 years, and can achieve an adult size up to 60 cm (Shepherd, 2000). They are omnivores and feed on crustaceans, mollusks, echinoderms, fish, as well as plants (Shepherd, 2000).

The black sea bass is important to both commercial and recreational fisheries (Able *et al.*, 1995), with the recreational landings comparable in magnitude to those from the commercial fishery (Shepherd, 2000). Commercial fishing gears used to target black sea bass are otter trawls and fish pots. North of Cape Hatteras, commercial landings remained fairly constant at ~ 2,600 metric tons (mt) per year from 1887 until 1948, then

increased dramatically in 1952 to 9,900 mt before declining to only 600 mt in 1971 (Shepherd, 2000). From 1980 to 1998 commercial landings averaged  $\leq 1,500$  mt per year (Shepherd, 2000). The stock currently has a low biomass level and is classified as overfished (Shepherd, 2000). In response to the apparent population declines, the Atlantic States Marine Fisheries Commission (ASMFC) has assumed management of black sea bass under the Summer Flounder, Scup, and Black Sea Bass Fisheries Management Plan, and has implemented gear restrictions, minimum fish sizes, a coastal commercial quota and a recreational harvest limit (Shepherd, 2000) in an effort to restore these populations.

The considerable demand for this high value fish, coupled with a decline in stock abundance, has stimulated investigations into the potential for black sea bass culture. Previous studies report that black sea bass are resilient when handled and transported, adapt readily to formulated feeds (Copeland *et al.*, 2002), and tolerate a wide range of temperatures and salinities (Berlinsky 2000). These qualities suggest that black sea bass are well-suited for aquaculture.

The southern flounder (*Paralichthys lethostigma*) is a member of the family Paralichthyidae that ranges from Albemarle Sound, North Carolina to northern Florida, and from Tampa Bay, Florida along the Gulf coast to southern Texas (Wenner *et al.*, 1990; Daniels, 2000; Benetti *et al.*, 2001). The discontinuous distribution in south Florida suggests that there may be two genetically distinct stocks (Daniels, 2000; Zhang, 2001). Southern flounder (SF) inhabit coastal bays, sounds, lagoons, and river systems from spring through fall, and are most abundant in mid to upper reaches of estuaries,

occasionally entering fresh water (Benetti *et al.*, 2001). Their diet consists primarily of shrimps and small fishes (Daniels, 2000).

Adults migrate offshore to spawn in late fall and winter, then immediately return to estuaries and rivers (Daniels, 2000). The larvae remain in offshore waters for 30 to 60 days to feed on zooplankton, then metamorphosis begins and the larvae are transported through inlets to estuaries (Daniels, 2000). Post-metamorphic individuals migrate up estuaries where they are hypothesized to remain until they reach sexual maturity at two years of age (Daniels, 2000). Essentially, these fish require full strength seawater during their larval phase, however, after metamorphosis is complete they are tolerant of low salinity environments.

Southern flounder are the largest (maximum size 9 kg) paralicthyid occurring in inshore waters of the South Atlantic and Gulf coasts, and represent an important recreational and commercial species (Wenner *et al.*, 1990). Pound nets and gillnets are the primary gear types used by commercial fisherman to catch this species.

Unfortunately in the past, it was difficult to decipher the commercial landings data since it combined southern flounder with sympatric species: summer flounder (*Paralichthys dentatus*) and gulf flounder (*Paralichthys albigutta*) (Wenner *et al.*, 1990). However, the most recent stock assessment for southern flounder in North Carolina shows that commercial landings increased from 907 mt per year in the early 1980's to a peak of 2223 mt in 1994, followed by a 34% decline to 1456 mt in 2000 (Armstrong, 2001). Approximately 80% of the total landings were female, since males do not generally achieve lengths of legal size (Anderson, 2001). Currently, the stock is depleted, and efforts are being made to develop fishing and management practices which will reduce

fishing mortality. Since the southern flounder fishery appears to be recruitment-driven, juvenile abundance data may be used to warn of poor year classes, thus preparing managers and fishermen to reduce effort for that year (Armstrong, 2001). The ability of southern flounder to grow in fresh or brackish water combined with their high market value make them promising candidates for aquaculture. In addition to their tolerance of low salinities, they tolerate a wide temperature range (Watanabe *et al.*, 2000; Watanabe *et al.*, 2001) and can be trained to eat pelleted food in culture.

Techniques for eliciting compensatory growth in BSB and SF could be used to increase the production efficiency of these species for aquaculture. From the perspective of a balanced energy budget, compensatory growth would require that energy be allocated preferentially toward growth at the expense of some other component (i.e. reproduction, locomotion, etc.). Alternatively, proportional allocation could be maintained but consumption increased. The ability of many fishes to compensate their growth rate suggests that, under normal conditions, growth is not maximized (Nicieza & Metcalfe, 1997; Schultz *et al.*, 2002; Sogard & Olla, 2002). Ecologists have assumed that growth is always maximized during the juvenile stage since reaching a large size early is thought to increase fitness (Lotka, 1922; Ware, 1982; Stearns 1992). Many characteristics of fish are size dependent, such as risk of predation, size of prey, starvation resistance, mate choice and fecundity (Zhu *et al.*, 2001). However, the capacity for fishes to engage in compensatory growth suggests there are trade-offs that constrain normal growth to a sub-maximal rate (Schultz *et al.*, 2002; Sogard & Olla, 2002). Potential adaptive value of a sub-maximal growth rate has been demonstrated in studies on the Atlantic silverside (*Menidia menidia*) which have focused on swimming

performance and vulnerability to predation (Billerbeck *et al.*, 2001; Lankford *et al.*, 2001). Both studies revealed that maximization of energy intake rate and growth rate increases a fish's vulnerability to predation, due to decreased locomotory abilities. Schultz *et al.* (2002) suggests that juvenile growth rate is therefore strategic, in that it may be optimized with respect to conflicting selective pressures.

Another area of interest regarding growth of juvenile fishes deals with energy, or lipid, allocation. Hence, an examination of fish experiencing different levels of deprivation may elucidate which stores are actively used by fish during periods when food-resources are lacking. Furthermore, proximal composition analysis may help to indicate the mechanism of compensatory growth by revealing how energy allocation changes in fish that are actively compensating.

To date, researchers are not certain how compensatory growth evolved or what the metabolic cause is for hyperphagia, although some scenarios which would favor selection for compensatory growth are presented. The advantage of a capacity to compensate growth rate is that individuals will have increased resiliency to adverse conditions for growth, and may recover from periods of reduced growth opportunity to a greater extent than individuals that do not engage in compensatory growth. For example, animals living in a seasonal environment repeatedly endure a wintertime period of reduced energy intake, followed by a springtime period of high energy availability and reproductive expenditure (Schultz *et al.*, 2002). For such animals, there should be strong selection favoring a rapid recovery from winter starvation or shorter term disturbances in resource availability (Broekhuizen *et al.*, 1994; Schultz *et al.*, 2002). For some species, such as *Menidia menidia*, there is a minimum threshold size that is critical for survival

through the winter (Conover and Present, 1990), thus a mechanism that would ensure that this target size is reached would be highly desirable. Likewise, for animals whose prey is distributed unevenly in space or time, periods of under-nutrition may be experienced with regularity. Hence, one would expect species that inhabit predictable environments, where food availability is generally high, to show less compensatory ability, whereas species that experience highly seasonal food availability or patchily-distributed resources would be more likely to evolve the capacity to compensate growth rate.

Black sea bass are reef-associated as adults, and after spending time in coastal areas as juveniles, they migrate out to offshore reefs which provide them with adequate habitat and food resources. By virtue of being away from shallow coastal areas, the temperature fluctuations of offshore reefs should be minimal, especially so in locations south of Cape Hatteras since the Gulf Stream would influence the waters there. Reefs also serve to attract higher numbers of individual organisms due to their topographical complexity which creates habitat for fish and other marine invertebrates. As such, food availability for black sea bass should remain high due to the productivity of reefs, and the higher numbers of fish associated with it.

Southern flounder are estuarine-dependent fish, and live high up in estuaries near the mouth of rivers, except during spawning periods when they move out to oceanic waters. Due to their habitat preference, the southern flounder must endure seasonal temperature variations as well as seasonal prey abundances. Thus, they are more likely than black sea bass to experience episodes of starvation during the course of a year.

The implications of compensatory growth for aquaculture are clear – successful exploitation of this naturally occurring mechanism should increase production rate and



minimize grow-out times. Understanding the dynamics of compensatory growth may allow for the design of feeding schedules to improve aquaculture production rate by maximizing growth rates (Zhu *et al.*, 2001) and/or growth efficiencies. Additionally, compensatory growth may be looked upon as a management tool for aquaculture. Besides increased feed efficiency and enhanced production in commercial aquaculture, the feeding strategies used to elicit compensatory growth may improve management of personnel time, water quality, and fish-feeding activity (Gaylord & Gatlin, 2001).

The objectives of this study were to 1) identify feeding regimes that would maximize the compensatory response of BSB and SF, 2) determine if compensatory growth is associated with changes in body composition, and 3) assess the feasibility of using compensatory growth in commercial scale aquaculture. In addition, the contrasts observed from these species were analyzed using an ecological life-history context to help explain the causes for any differences seen. Finally, an evaluation was made of the technique that was used during this study to elicit compensatory responses.

## METHODS

### Subject Animals

Experimental subjects were obtained as early juveniles from hatchery-reared stocks at the UNCW Aquaculture Facility at Wrightsville Beach, NC. Broodstock, of local origin, of both black sea bass and southern flounder are maintained at the facility under a controlled photo-thermal cycle, and were the source of juveniles used in these compensatory growth experiments. At approximately 60 days post hatch for BSB and 90 days post hatch for SF, the juveniles were collected from the UNCW and Virginia Tech

aquaculture facilities, respectively, then transported to the wet lab at the Center for Marine Science (CMS) where the experiments were conducted. Upon arrival at the CMS wet lab, the fish were fed a 55% protein/ 10% lipid 1mm sinking feed (Biokyowa: Chesterton, Missouri, USA) which was found by Berlinsky et al. (2000) to promote superior growth over other commercial feeds. The salinity of the water was gradually reduced from full strength seawater to 20 parts per thousand (‰) over the period of 3 days. Once these conditions were reached, each fish was randomly allocated to an individual 40-liter aquarium and given 10 days to acclimatize to its surroundings while fed to satiation daily.

### System Design

Experiments were carried out in a fully-recirculating system consisting of sixty 40-liter aquaria, and three 160 liter sump tanks. Also, two 600 liter tanks were used, one to hold the group control fish for the BSB experiment (see below), and one that served for mixing artificial seawater (using Instant Ocean) that was used throughout the experiment. All the tanks were kept under controlled conditions. For both black sea bass and southern flounder, the water temperature and salinity were maintained at 23 °C and 20‰, respectively. These conditions have been reported to maximize growth rate in both species (Berlinsky, 2000; U. Howsen, pers. comm.). Photoperiod was controlled at 12L:12D, and aeration was provided by air stones placed in each of the aquaria. Removal of wastes and uneaten feed was done by siphoning the tanks daily. Water removed during siphoning (~10%) was replaced with clean, pre-mixed salt water each day.

## Experimental Design

In order to study the compensatory growth response, subjects were divided into 5 groups: 2 control groups [one group control (GC), and one individual control (IC)] which were fed to satiation twice daily throughout the experiment, and 3 treatment groups termed D2, D5, and D8 which were deprived of food for 2, 5, and 8 consecutive days, respectively (Table 1). These treatment groups were designed to provide three different levels of deprivation: the D2s represent mild deprivation, the D5s represent moderate deprivation, and the D8s represent severe deprivation. Group controls were held differently in the two experiments: for BSB, the GC fish (n=23) were held in a 600 liter tank filled with approximately 250 liters of water, and for SF the GC fish (n=20) were divided among four 10 liter tanks with 5 fish held per tank. The fish chosen for each of the experiments were selected so that the mean weights and lengths at the beginning of the study were not significantly different across the 5 treatments (Table 1).

The growth experiment was conducted in two consecutive phases using different protocols. For each phase, the intent was to evaluate the growth, food consumption, and growth efficiency of the treatment groups and compare them to the individual control group. During Phase I, compensation of treatment groups compared to the controls was tested under a cyclical feeding regime. For example, the D5 fish were deprived 5 consecutive days, and then fed to satiation twice a day until their hyperphagic response waned (weight-specific consumption was not significantly higher than the IC for 2 consecutive days), at which time they were deprived again for 5 days. The duration of Phase I was sufficient to ensure that each of the treatment groups had cycled through their deprivation- refeeding schedule at least three times.

Table 1. Experimental design of study showing feeding regimes for treatments during Phase I and Phase II.

<b>Treatments</b>	<b>Initial Mean Wet Weight (g) (S.E)</b>	<b>Initial Mean Length (mm) (S.E.)</b>	<b>N</b>	<b>Feeding Regime (Phase I)</b>	<b>Feeding Regime (Phase II)</b>
<b>Group Control</b>	BSB = 2.54 (.20) SF = 3.70 (.29)	BSB = 54.83 (1.25) SF = 79.75 (1.75)	BSB = 1 (23 individuals) SF = 4 (5 individuals/rep)	2 x per day (ad libitum)	2 x per day (ad libitum)
<b>Individual Control</b>	BSB = 2.61 (.21) SF = 4.58 (.38)	BSB = 55.80 (1.45) SF = 81.00 (1.76)	BSB = 15 SF = 14	2 x per day (ad libitum)	2 x per day (ad libitum)
<b>D2</b>	BSB = 2.61 (.16) SF = 4.04 (.36)	BSB = 55.67 (1.08) SF = 79.93 (2.16)	BSB = 15 SF = 14	Deprived 2 consecutive days, then re-fed	2 x per day (ad libitum)
<b>D5</b>	BSB = 2.95 (.27) SF = 4.29 (.32)	BSB = 57.73 (1.65) SF = 82.57 (1.47)	BSB = 15 SF = 14	Deprived 5 consecutive days, then re-fed	2 x per day (ad libitum)
<b>D8</b>	BSB = 2.76 (.24) SF = 4.17 (.37)	BSB = 56.33 (1.47) SF = 81.57 (1.69)	BSB = 15 SF = 14	Deprived 8 consecutive days, then re-fed	2 x per day (ad libitum)



Phase II was used to test whether the deprived fish could catch up to controls if given unlimited food. During this phase, all treatments were fed twice daily to satiation. As a consequence of Phase I, the groups of fish represented 4 different levels of deprivation: IC & GC = no deprivation, D8 = severe deprivation, and D2 & D5 = intermediate levels of deprivation. Thus, it was possible to determine whether compensatory growth was initiated by some threshold level of deprivation. After Phase II was completed, all of the individuals were sacrificed using a lethal dose of MS-222, and stored at -80°C for proximal composition analysis. All experimental protocols employed during this study were reviewed and approved by the UNCW Institutional Animal Care and Use Committee (protocol # 2002-008).

#### Growth

Individual fish were weighed ( $\pm 0.001$  g) on a Navigator scale and measured for total length to the nearest millimeter, at the start of the experiment and at biweekly intervals thereafter. A 24-hour fast was implemented prior to all weight measurements to allow for the elimination of gut contents. Also, excess water was blotted from individuals using lint-free towels prior to weighing. Individual growth rates for each interval were calculated as specific growth rate (SGR):

$$\text{SGR} = (\ln W_f - \ln W_i) / T \times 100\%,$$

where,  $W_f$  is final weight,  $W_i$  is initial weight, and T is time.

#### Consumption

Two different sizes of pelleted food were used during this study. For the entire BSB experiment and PI of the SF experiment, fish were fed pellets weighing 0.0542 g (coefficient of variation = 13%), and due to logistical constraints during PII of the SF

experiment a larger pellet was fed to the fish weighing 0.01571 g (coefficient of variation = 20%). Pellet integrity was very high, and they did not dissolve or disintegrate in the tanks before any of the uneaten pellets were removed at the end of the day. Thus, the food pellets given to each fish were counted at each feeding, and before siphoning, the number of pellets remaining were tallied so that the weight of food actually consumed per day by each fish could be calculated. Weight specific feeding rate (WSFR) was used to assess hyperphagia and to determine the initiation of a new deprivation period:

$$\text{WSFR} = \text{g food consumed} / \text{g fish}.$$

Weights of the fish were estimated for the days between actual weighings by assuming exponential growth:

$$W_t = W_0 e^{Gt}$$

where,  $W_t$  is weight at time  $t$ ,  $W_0$  is weight at time 0, and  $G$  is the instantaneous growth coefficient. At the end of the study, the daily WSFR values were corrected to take into account the weights of the fish after they were determined using the equation above.

Gross growth efficiency ( $K_1$ ) was calculated for each individual, according to:

$$K_1 = (W_f - W_i) / CC,$$

where,  $W_f$  is final weight,  $W_i$  is initial weight, and  $CC$  is cumulative consumption of food in grams during the growth interval.

#### Proximate Composition

Proximate composition of fish tissues was examined to assess changes in water, lipid, and protein content of individual fish in response to the deprivation treatments.

Dissections were performed to remove the liver and viscera of each fish, then wet weights were recorded immediately. The tissues were then dried at 60°C for 48 hours to

determine water content. Following this procedure, non-polar lipids were extracted using petroleum ether and quantified gravimetrically (Schultz & Conover, 1997) (see Appendix). Visceral tissue was extracted separately from the carcass and liver. Visceral tissue was omitted from the ashing process due to the method in which the visceral DWT was obtained – the lipid-rich viscera had to be wiped out of the drying pans and in doing so the tissues were mixed in with lint-free towels, and could not be easily separated out in order to be ashed. After the soxhlet extractions, the remaining lean tissue was considered to represent the protein content of the tissue, assuming carbohydrate within the tissue was negligible. Additionally, the hepatosomatic index (HSI) was calculated on a wet and dry weight basis as:

$$\text{HSI} = \text{g liver} / \text{g fish}$$

The HSI values were used to determine whether fish exhaust lipid reserves from their livers during compensation.

#### Ashing

The ash free dry weight (AFDW) was determined in order to calculate the organic composition of the lean tissue. Pre-ashed crucibles were prepared prior to ashing by placing them in a muffle furnace at 450°C for 24 hours. The weight of these crucibles was recorded before a tissue sample was homogenized and placed in each one. The crucible was then re-weighed so the pre-ashed weight of the tissue sample could be recorded. The crucibles were placed into the furnace for 24 hours at 450°C. The ashed weight was recorded for each crucible after they were kept for 24 hours in a 60°C drying oven, and calculations were done to determine the AFDW for each of the tissue samples.



## Null Hypotheses

H<sub>0</sub> 1 – Cyclical feeding will not enhance growth rates of juvenile black sea bass and southern flounder relative to control fish fed ad libitum.

H<sub>0</sub> 2 – Fish exhibiting compensatory growth will not differ in body composition (protein, lipid, water) from control fish.

H<sub>0</sub> 3 – Fish inhabiting highly seasonal environments with patchily distributed food resources (southern flounder) will not display a greater capacity for compensatory growth than fish that experience less variation in food resources (black sea bass).

## Analysis

The feeding data for every day of PI were analyzed using a student's T test to determine whether the mean consumption (WSFR) of each treatment group was significantly higher than the individual control group (in order to detect a hyperphagic response). The growth rate data were analyzed for both of the controls and the different treatments using a repeated measures ANOVA using interval growth data as blocks in the analysis, and post-hoc Tukey's HSD multiple comparison test. To adjust for size, ANCOVAs were used both within species and across species. The proximal composition data were analyzed using a one-way ANOVA for each of the parameters (protein, lipid and water). The hepatosomatic index data and ashing data were also analyzed using one-way ANOVAs. An alpha value of 0.05 was used in all statistical analyses. A Levenes test was used to determine the homogeneity of variances, and data sets were transformed using the arcsin-square root transformation (BSB PI WSFR) and the Log<sub>10</sub> transformation (SF Lengths and SF Weights) in order to meet this requirement.

## RESULTS

### Phase I Black Sea Bass

#### Consumption

During PI of the experiment, when the cyclical deprivation/re-feed regime was imposed on the D2, D5, and D8 treatments, a repeated measures ANOVA showed that WSFR was influenced significantly by both treatment and interval (treatment:  $F = 19.476$ ,  $P < 0.001$ ; interval:  $F = 646.829$ ,  $P < 0.001$ ). There was also a highly significant interaction (interval\*treatment) for WSFR ( $F = 27.551$ ,  $P < 0.001$ ). The significant interaction term arose due to the drop in WSFR for D8 during the second interval of PI (Figure 1), while the IC, D2 and D5 treatments showed an increasing WSFR during that same period. The mean WSFR values for treatments were all significantly lower than the IC fish, and the most deprived treatment, D8, had the smallest WSFR value. The other treatments, D2 and D5, had WSFR values that reflected the amount of deprivation they experienced in PI, and they fell in between the IC and D8 groups. Notably, the hyperphagic response of the treatments upon refeeding was short-lived and only lasted for one day for all treatments regardless of deprivation time (Figure 2).

Cumulative consumption (CC) for BSB during PI showed a similar stepwise decrease as WSFR. The average consumption of each fish in the IC group was 8.093 g ( $\pm 0.747$  g) of food, and the D2 (4.533 g  $\pm 0.5$  g), D5 (2.727 g  $\pm 0.286$  g), and D8 (1.488 g  $\pm 0.195$  g) treatments followed with respectively less food consumed on average per fish.

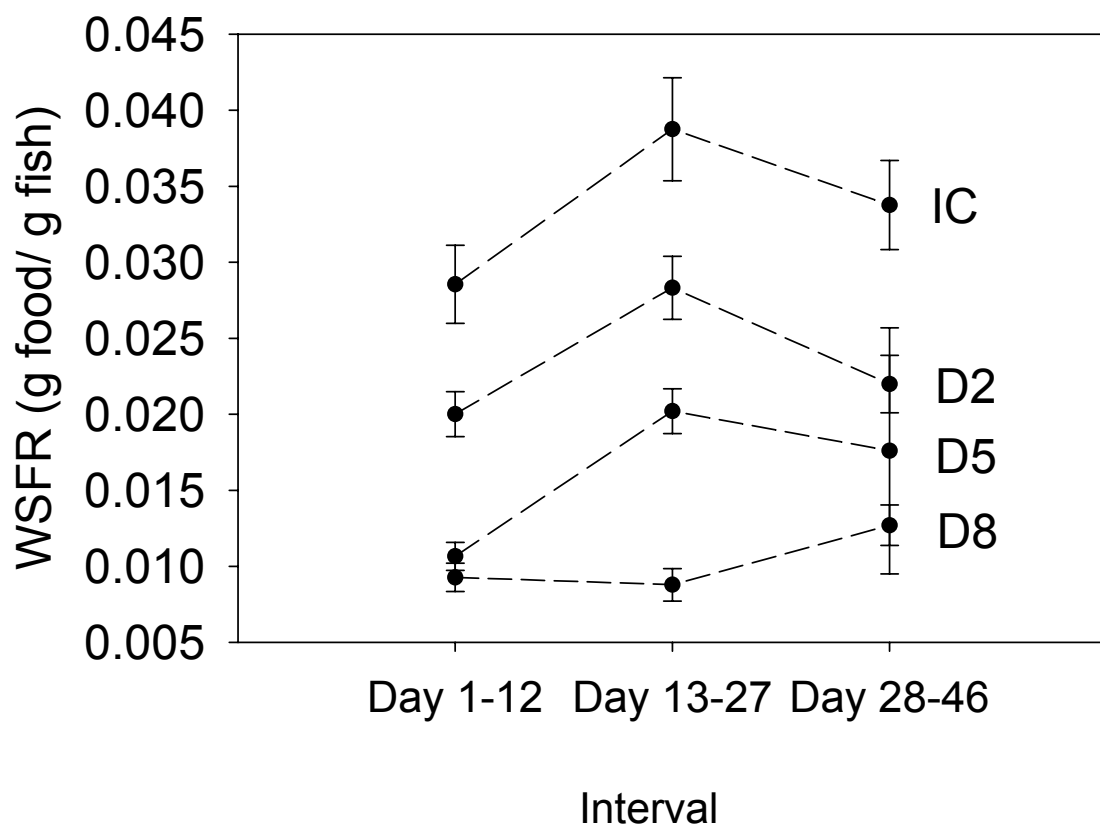


Figure 1. Average weight specific feeding rate (WSFR) values for black sea bass treatments for each interval during PI. Vertical bars indicate  $\pm 1$  standard error.

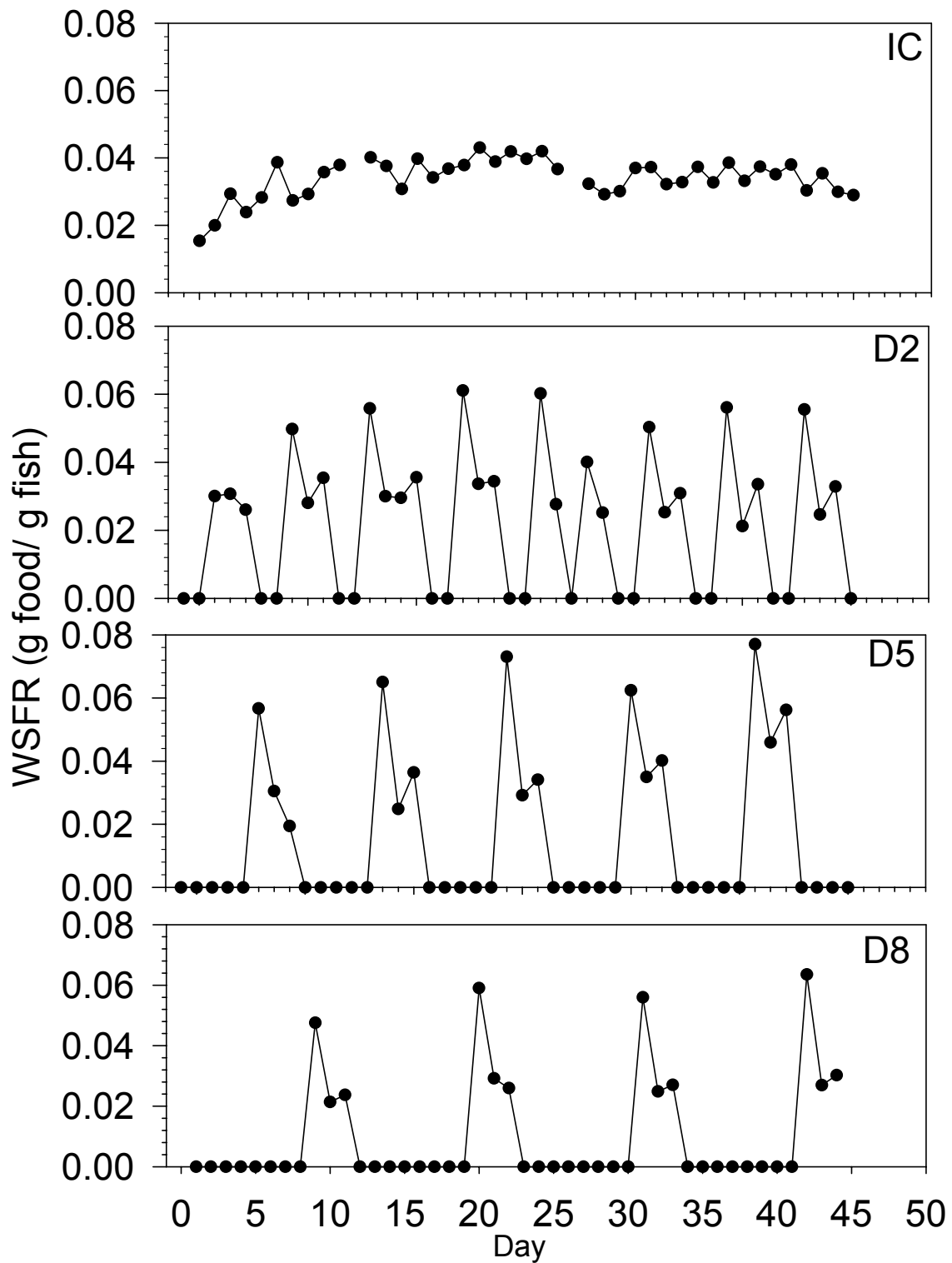


Figure 2. Daily averaged weight specific feeding rate for each treatment of black sea bass during PI. Data gaps for IC group represent fasting periods imposed prior to weight measurements.

## Growth

Growth was measured in terms of wet weight (WWT) and total length (TL), and these parameters responded similarly to treatments during PI. Both parameters showed significant interactions (interval\*treatment) indicating that the treatments affected growth in different ways (TL:  $F = 21.655$ ,  $P < 0.001$ ; WWT:  $F = 31.391$ ,  $P < 0.001$ ). In both cases, the IC grew faster than the other treatments, as evidenced by a much steeper slope on the growth curve than the D2, D5, and D8 groups (Figure 3). Controls maintained a size advantage throughout PI in both WWT and TL, and the remaining treatments followed respectively smaller as their deprivation time increased. These findings indicate that the cyclical feeding regimes employed during PI did not elicit a strong compensatory growth response.

Growth rates during PI were significantly different across the treatments ( $F = 27.583$ ,  $P < 0.001$ ). The IC maintained higher SGR values than the treatments throughout PI (Figure 4). There was also a highly significant interaction term (interval\*treatment) for growth rate in PI ( $F = 9.330$ ,  $P < 0.001$ ), which was due to the D8 treatment not increasing its SGR value during the second interval of PI while the rest of the treatments experienced similar increases in growth rate during that time. Also, the D8s increased SGR during the last interval of PI while the other treatments had decreasing SGR values.

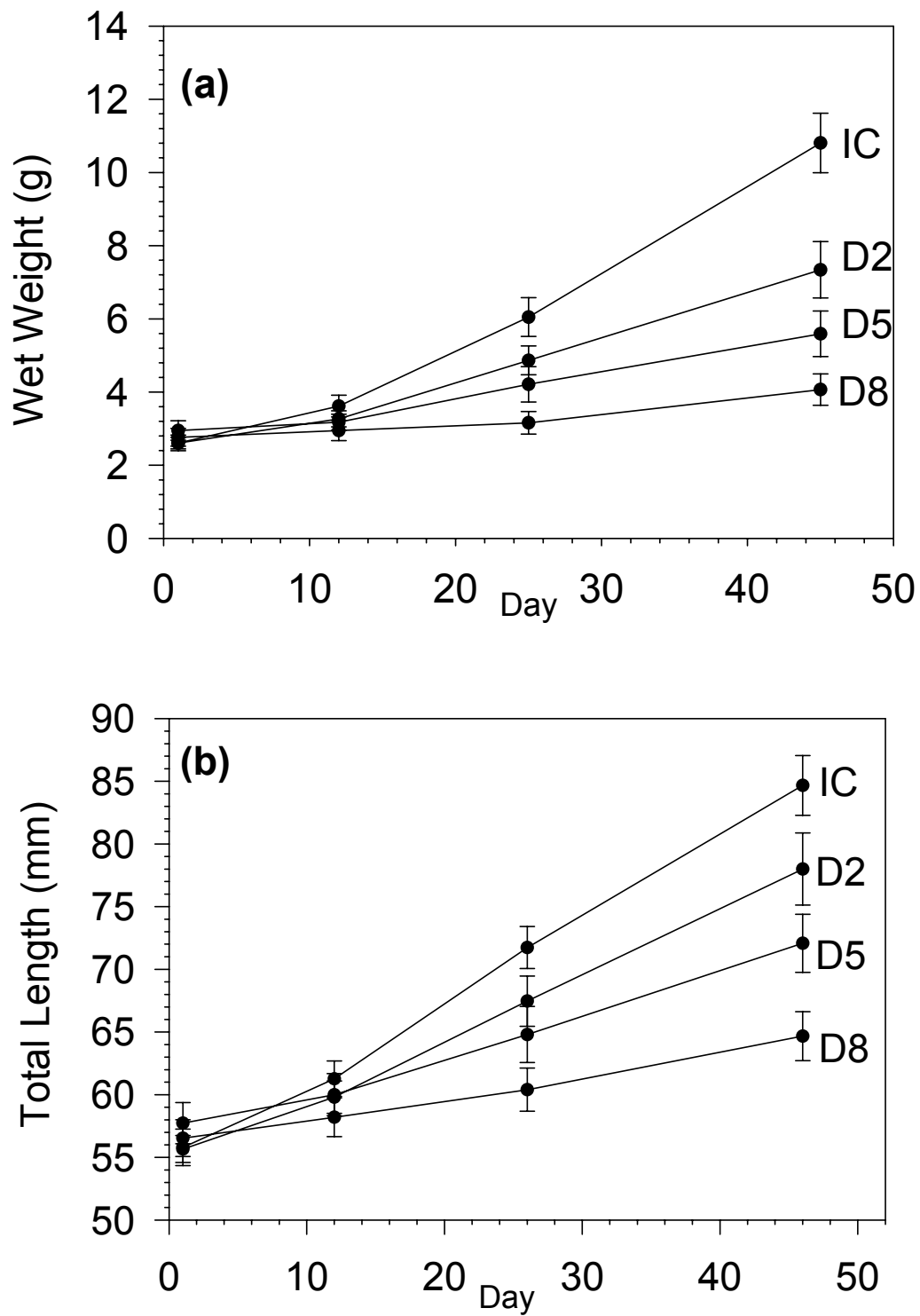


Figure 3. Averaged wet weight growth curve (a) and total length growth curve (b) for black sea bass treatments during PI. Vertical bars represent  $\pm 1$  standard error.

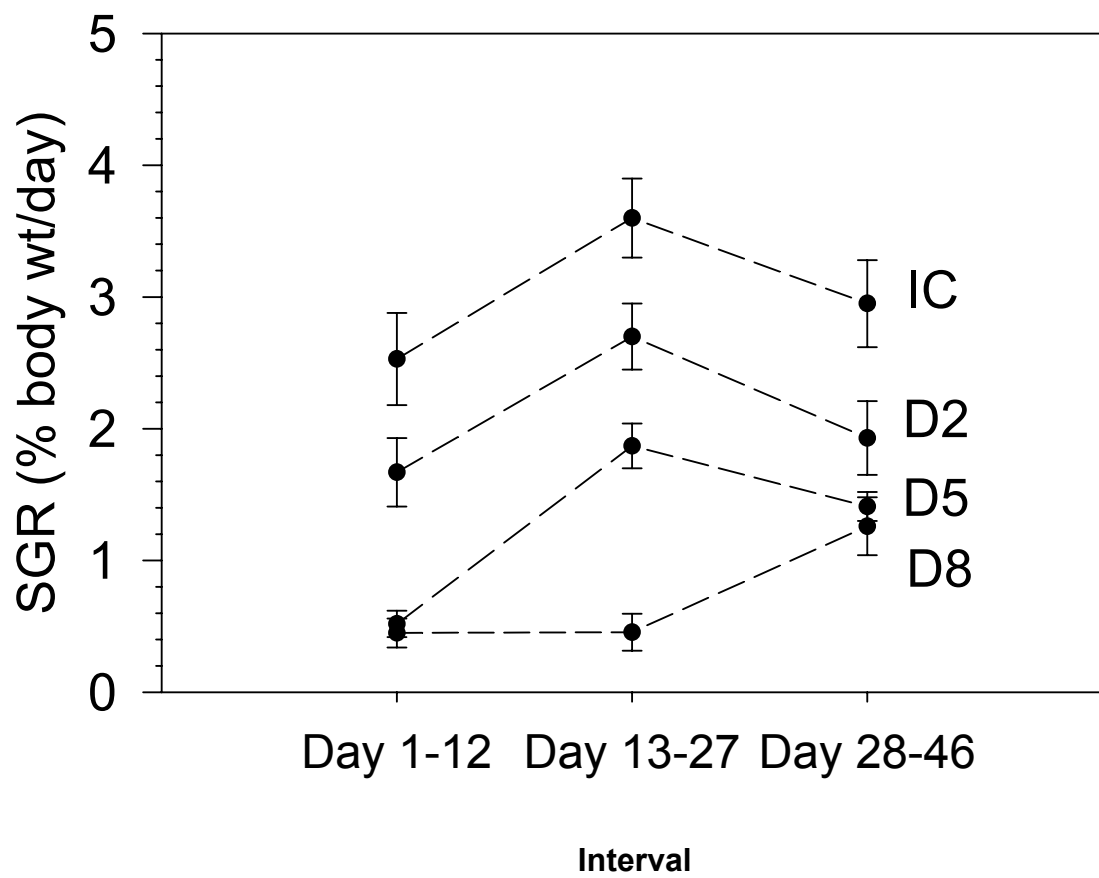


Figure 4. Mean specific growth rate values of black sea bass treatments for each interval of PI. Vertical bars represent  $\pm 1$  standard error.

### Gross Growth Efficiency ( $K_1$ )

Gross growth efficiencies were not significantly different among treatments during PI ( $F = 1.554$ ,  $P = 0.211$ ). The IC, D2, and D5 treatments had very similar  $K_1$  values, 0.898, 0.903, and 0.762 g fish growth/g food respectively, the D8 treatment showed a lower  $K_1$ , -0.217 g fish growth/g food, for PI (Figure 5). The negative value indicates that the D8 fish lost weight during PI.

### Phase I Southern Flounder

#### Consumption

During PI the SF treatments reacted in a similar way as the BSB to the cyclical deprivation/re-feed regime. Both main effects, treatment and interval, were highly significant (treatment:  $F = 24.153$ ,  $P < 0.001$ ; interval:  $F = 0.00008$ ,  $P < 0.001$ ), and there was a significant interaction term (interval\*treatment) for WSFR ( $F = 2.863$ ,  $P = 0.013$ ). The difference in the interaction term reflects the variable WSFR values among the treatments during the intervals of PI (Figure 6). Mean WSFR of IC and D2 treatments were not significantly different; however, both were significantly higher than the D5 and D8 treatments. As with the BSB, the WSFR values decreased with increased deprivation times. Again, the hyperphagic response was limited in duration upon re-feeding during PI (Figure 7).

The SF showed a decrease in CC with increasing deprivation time. The IC fish consumed a total of  $7.465 \text{ g} \pm 0.54 \text{ g}$  of food per fish, and the D2 ( $4.869 \text{ g} \pm 0.457 \text{ g}$ ), D5 ( $1.230 \text{ g} \pm 0.066 \text{ g}$ ), and D8 ( $0.773 \text{ g} \pm 0.06 \text{ g}$ ) treatments had successively lower CC values.



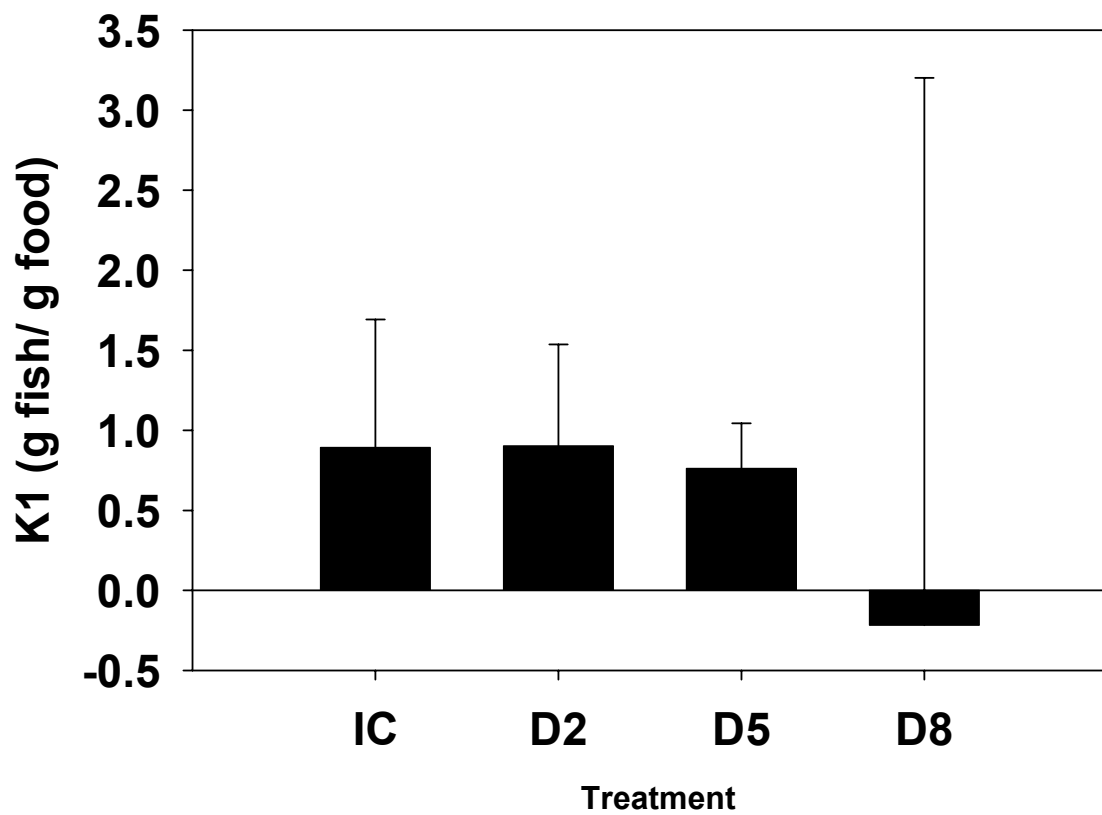


Figure 5. Mean gross growth efficiency values ( $K_1$ ) for black sea bass treatments during PI. Vertical bars represent  $\pm 1$  standard error.

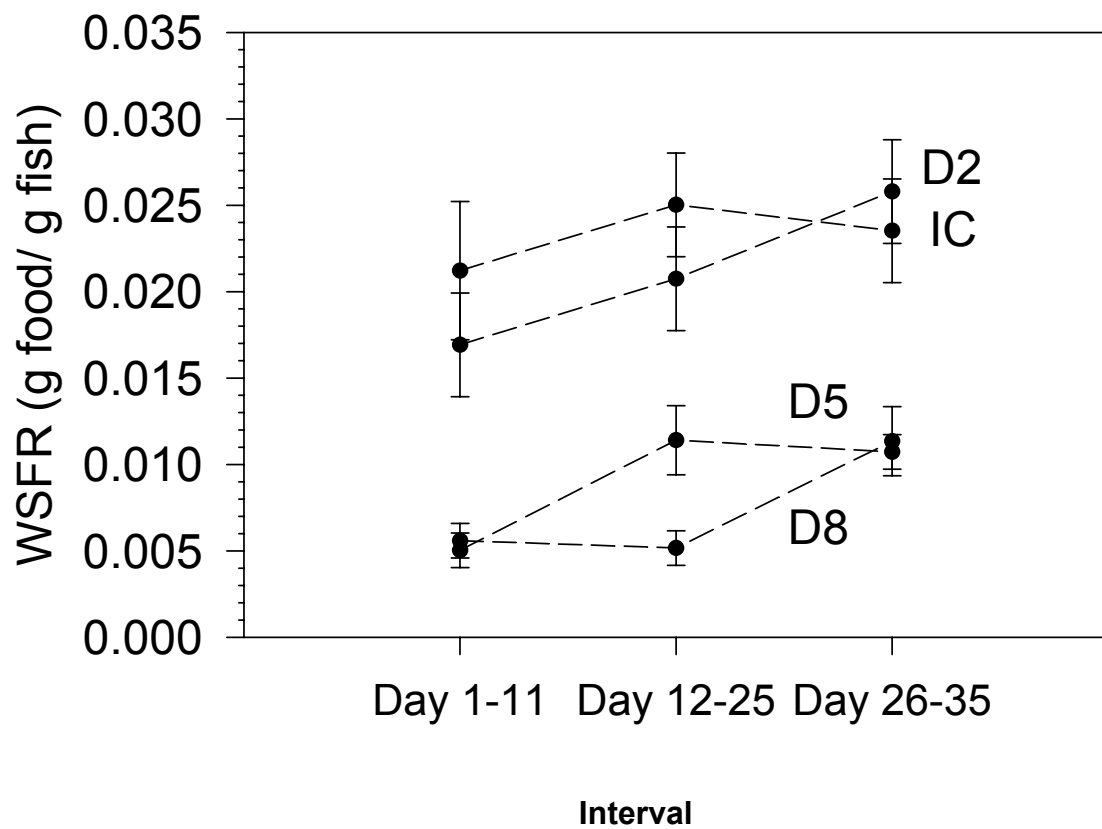


Figure 6. Mean weight specific feeding rate (WSFR) values for southern flounder treatments for each interval of PI. Vertical bars represent  $\pm 1$  standard error.

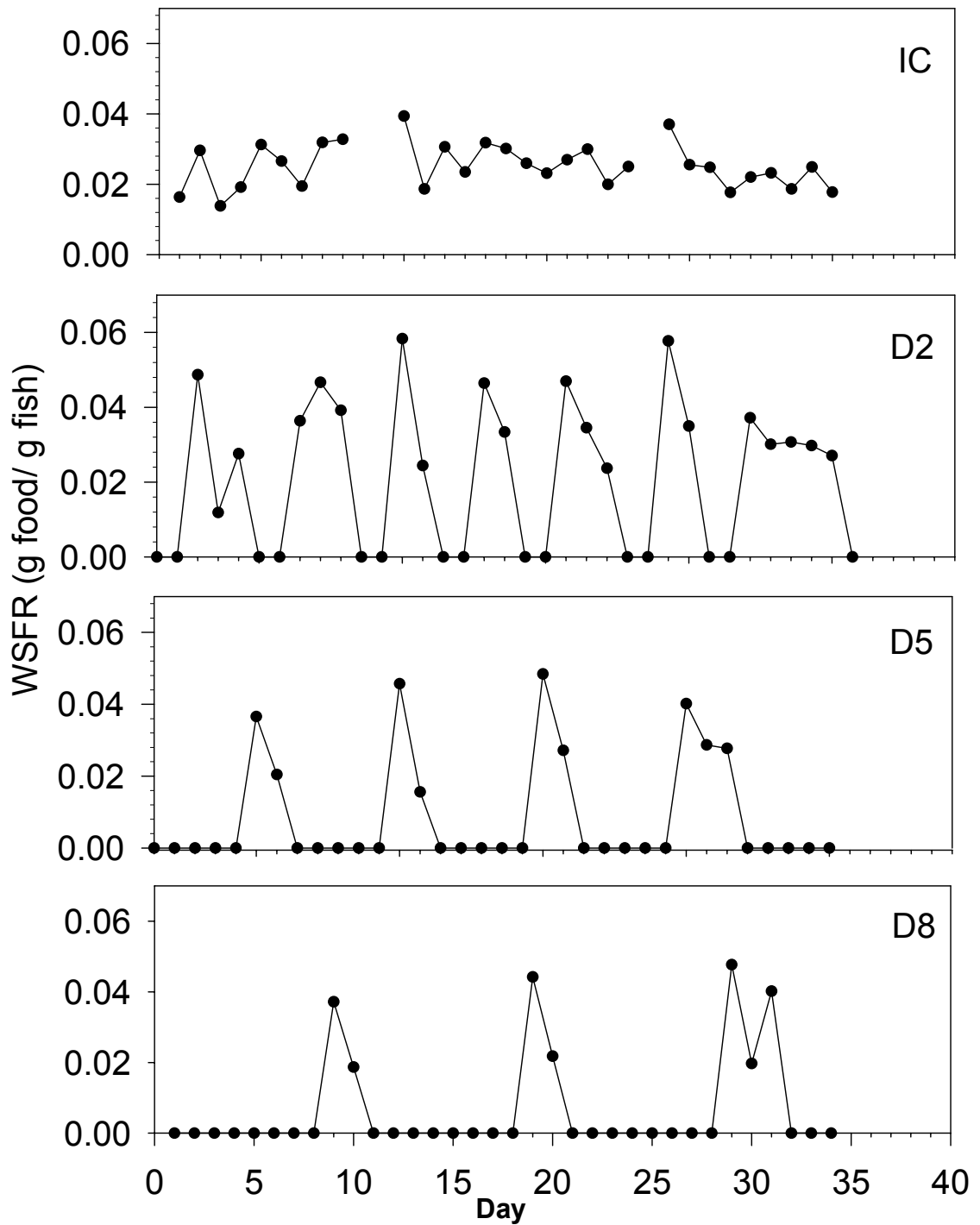


Figure 7. Daily mean weight specific feeding rate (WSFR) values for southern flounder treatments during PI. Data gaps for IC group represent fasting periods imposed prior to weight measurements.

## Growth

The TL and WWT of SF responded similarly to treatments during PI, and showed significant interaction terms (interval\*treatment) indicating that treatments behaved differently through time (TL:  $F = 20.346$ ,  $P < 0.001$ ; WWT:  $F = 21.173$ ,  $P < 0.001$ ). The IC fish grew faster in both length and weight than the other treatments (Figure 8). Again, the IC fish increased in size throughout PI in terms of TL and WWT, while the other treatments followed respectively smaller as their deprivation time increased.

Growth rates of SF during PI differed across treatments ( $F = 10.093$ ,  $P < 0.001$ ), however unlike the BSB, there was no significant interval\*treatment interaction. The D5 and D8 treatments had much lower SGR values than the IC and D2 treatments during each interval of PI (Figure 9). As seen previously, the treatments displayed the pattern seen in other analyses:  $IC > D2 > D5 > D8$ .

## Gross Growth Efficiency ( $K_1$ )

Gross growth efficiency of SF differed significantly among treatments ( $F = 3.347$ ,  $P = 0.029$ ) with the D5 treatment,  $-2.957$  g fish/g food, having a significantly lower  $K_1$  than D2 fish,  $1.648$  g fish/g food ( $P = 0.04$ ). None of the treatments were significantly different from the IC,  $1.368$  g fish/g food (Figure 10). However the D5 and D8 ( $-0.708$  g fish/g food) treatments were both negative, indicating a loss of weight during PI.

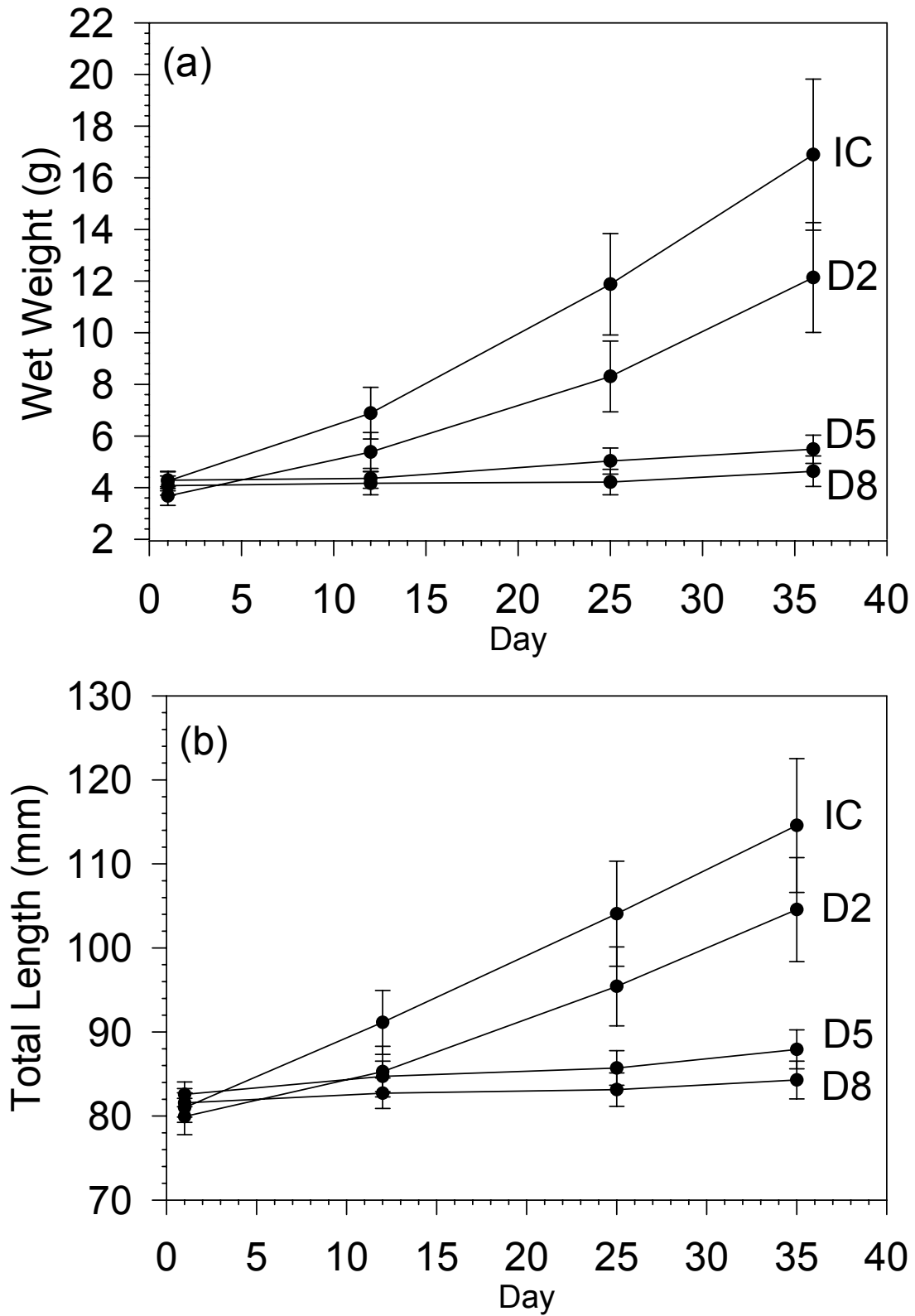


Figure 8. Mean wet weight growth curve (a) and mean total length growth curve (b) for southern flounder treatments during PI. Vertical bars represent  $\pm 1$  standard error.

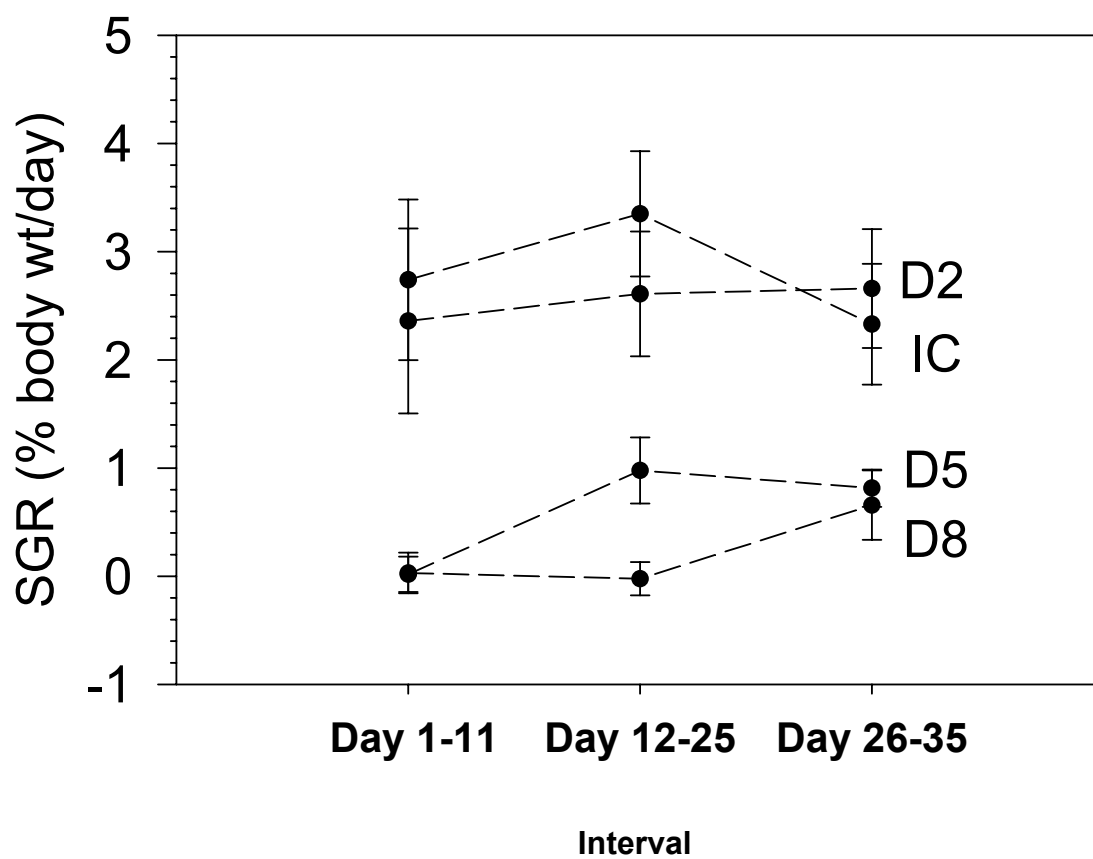


Figure 9. Mean specific growth rate values for southern flounder treatments for each interval during PI. Vertical bars represent  $\pm 1$  standard error.

## Phase II Black Sea Bass

### Consumption

During PII, when all the treatments were fed twice daily to satiation, an ANCOVA showed that WSFR of black sea bass covaried negatively with WWT ( $F = 9.377$ ,  $P = 0.003$ ). Size-adjusted WSFR of D5 and D8 subjects were significantly greater than those for D2 and IC subjects ( $F = 9.853$ ,  $P < 0.001$ ). Thus, in PII the rank order of WSFR for black sea bass is opposite from the results after PI, suggesting a hyperphagic response by D5 and D8 fish (Figure 11).

The CC for PII shows that there is a significant treatment effect ( $F = 13.529$ ,  $P < 0.001$ ). Total CC in grams for PII was as follows: IC =  $8.752 (\pm 1.17 \text{ g})$ , D2 =  $6.541 (\pm 1.075 \text{ g})$ , D5 =  $7.823 (\pm 1.119 \text{ g})$ , and D8 =  $5.976 (\pm 0.947 \text{ g})$ . Both D2 and D8 fish ate significantly less than IC fish, while the D5 fish were not different than the IC, and ate significantly more than the D2 and D8 treatments. Thus, D5 subjects appeared to increase their consumption during PII, exceeding that of the D2's which were not deprived as severely.

### Growth

In terms of somatic growth, the IC was able to maintain a size advantage throughout PII with respect to TL and WWT (Figure 12). There was a significant treatment effect on TL ( $F = 6.4$ ,  $P < 0.001$ ) with the IC having significantly greater lengths than the D8 treatment at the end of PII. Notably, the final total length of the D5 group was 87.86 mm which was slightly larger, however, not significant from the D2 final length of 87.20 mm. Similarly, there was a significant treatment effect on WWT

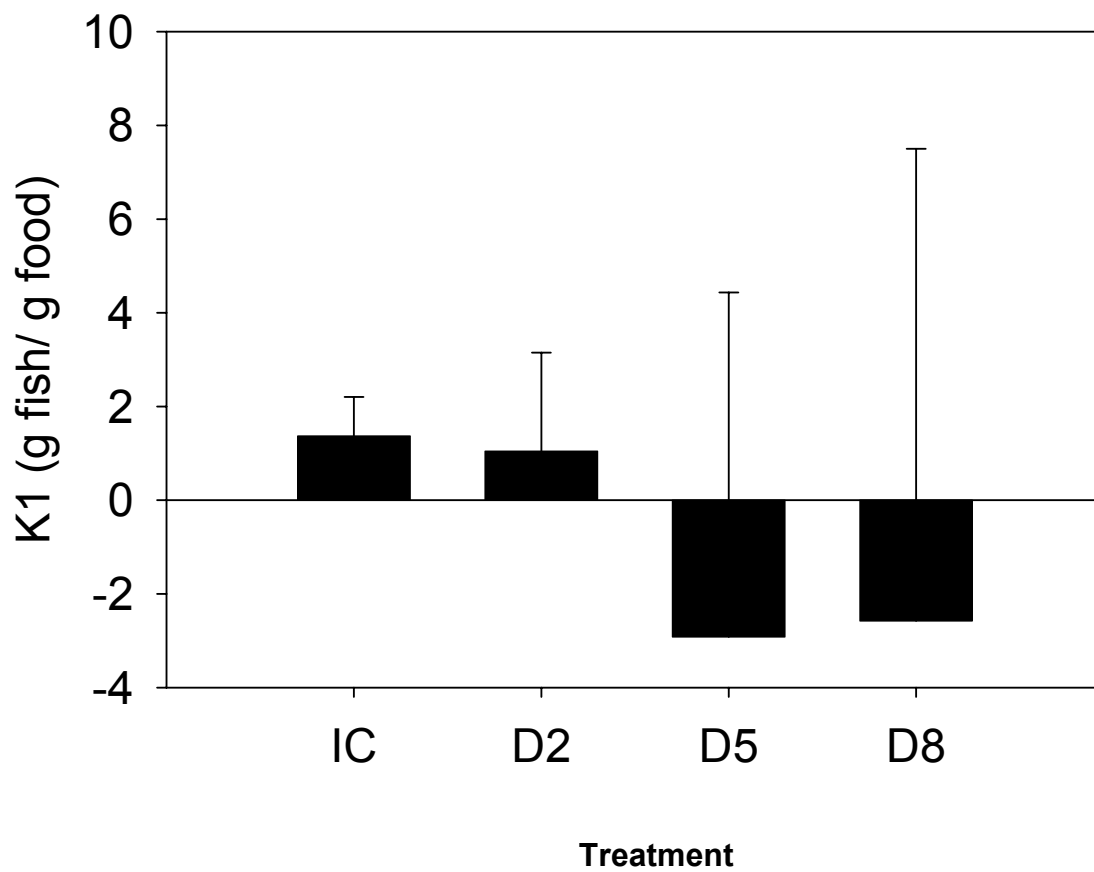


Figure 10. Mean gross growth efficiency values ( $K_1$ ) for southern flounder treatments during PI. Vertical bars represent  $\pm 1$  standard error.



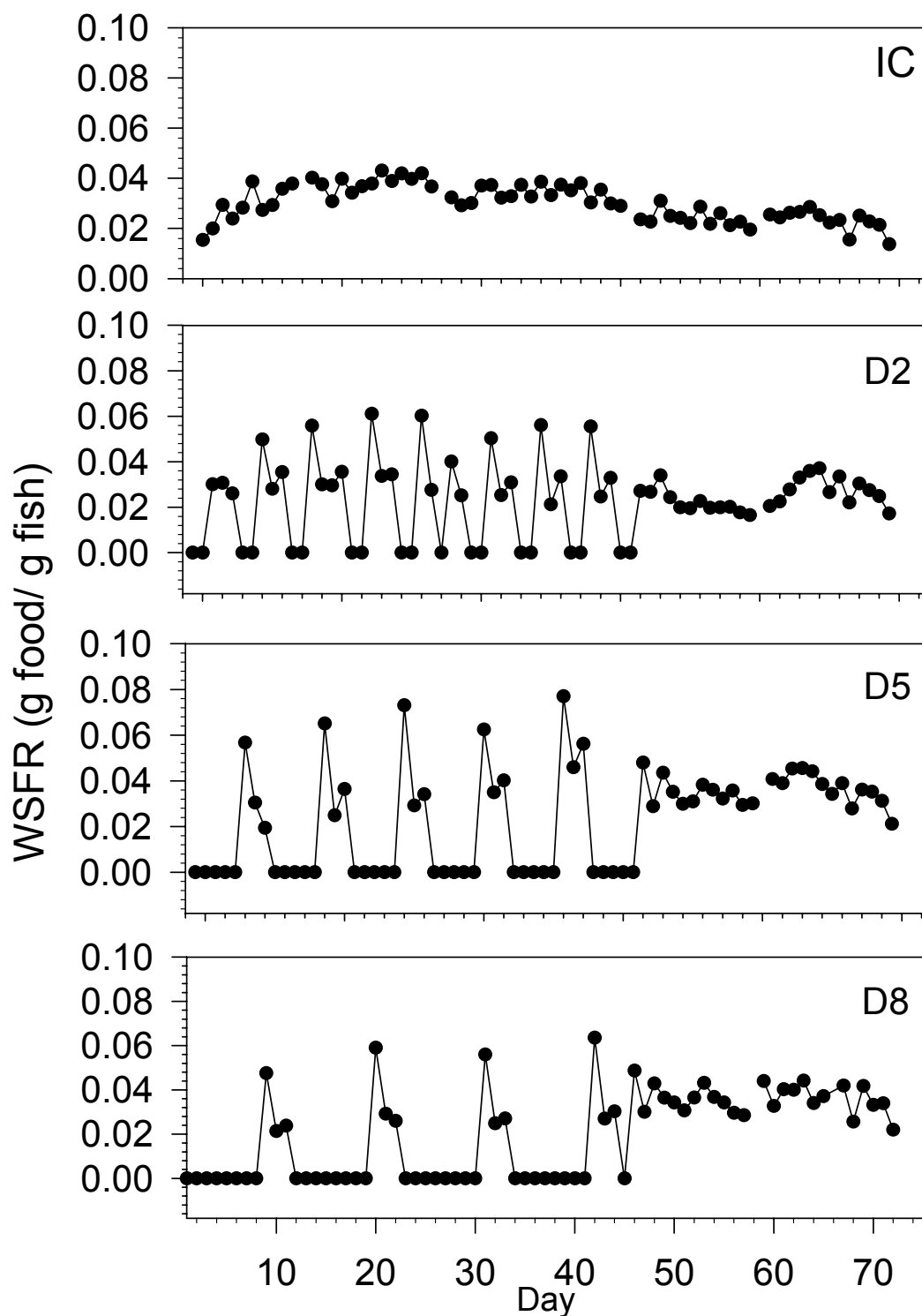


Figure 11. Daily mean WSFR values for BSB treatments during PI and PII. Data gaps for IC group represent fasting periods imposed prior to weight measurements.

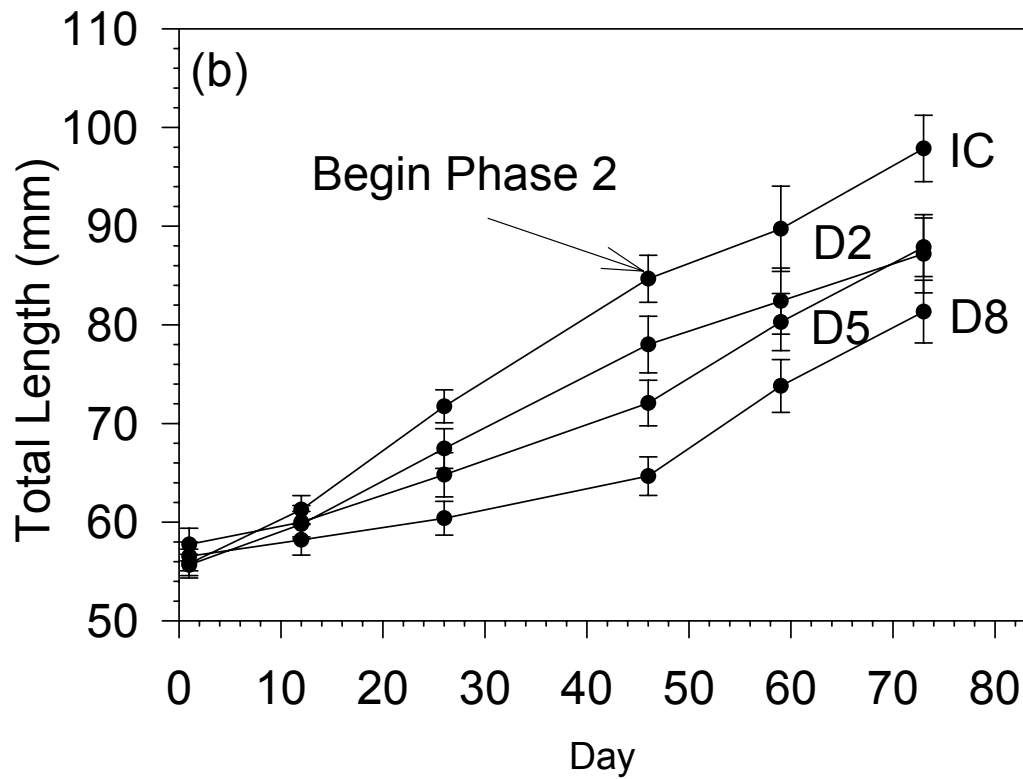
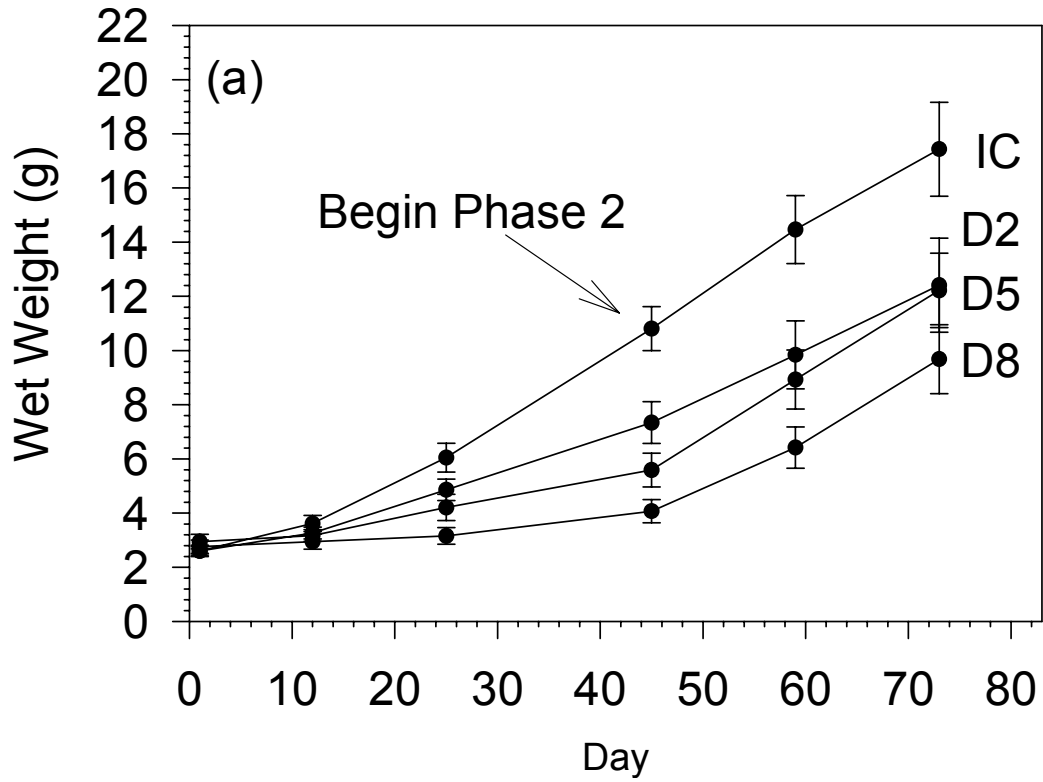


Figure 12. Average wet weigh growth curve (a) and total length growth curve (b) for black sea bass treatments during PI and PII. Vertical bars represent  $\pm 1$  standard error.

during PII ( $F = 8.648$ ,  $P < 0.001$ ). Again, the IC weighed significantly more than the D8 fish at the completion of PII, however D5s had a final WWT of 12.221 g which was only slightly less than the D2 final WWT of 12.411 g. Throughout PII, the D5 treatment narrowed the gap for both total length and WWT after PI when compared to the D2 treatment.

Growth rates of the BSB in PII showed a significant treatment effect ( $F = 5.866$ ,  $P < 0.001$ ), with the D8s having a significantly higher SGR value than the IC (Figure 13). Both the D5 and D8 treatments accelerated their growth rates in PII above the IC and D2 treatments.

#### Gross Growth Efficiency ( $K_1$ )

Gross growth efficiency did not differ among treatments during PII ( $P = 0.126$ ); however, there was a tendency for the most deprived treatments to exhibit higher  $K_1$  values (Figure 14). Thus the rank order for the treatments in terms of  $K_1$  is:  $D8 > D5 > D2 > IC$ .

### Phase II Southern Flounder

#### Consumption

Unlike the black sea bass, the southern flounder WSFR did not covary with WWT ( $F = 2.609$ ,  $P = 0.113$ ). There was a significant treatment effect on WSFR during PII ( $P = 0.012$ ), in which the D8 treatment had a significantly higher WSFR value than the IC (Figure 15). Although not significant, the D5 fish also showed a higher mean WSFR during PII. In contrast to PI, the trend for WSFR of southern flounder in PII was as follows:  $D8 > D5 > D2 > IC$ . Like the black sea bass, this increase in WSFR of the most deprived treatments indicates that a low-grade hyperphagic response was elicited in PII.

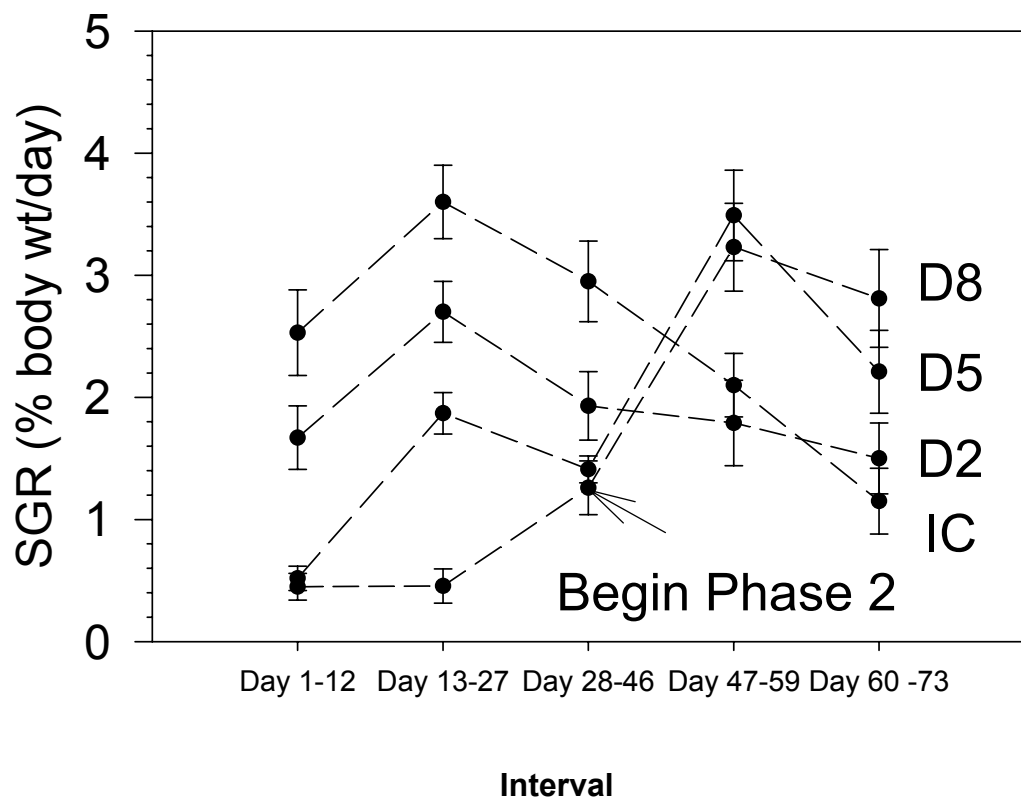


Figure 13. Mean specific growth rate values for black sea bass treatments per interval of PI and PII. Vertical bars represent  $\pm 1$  standard error.

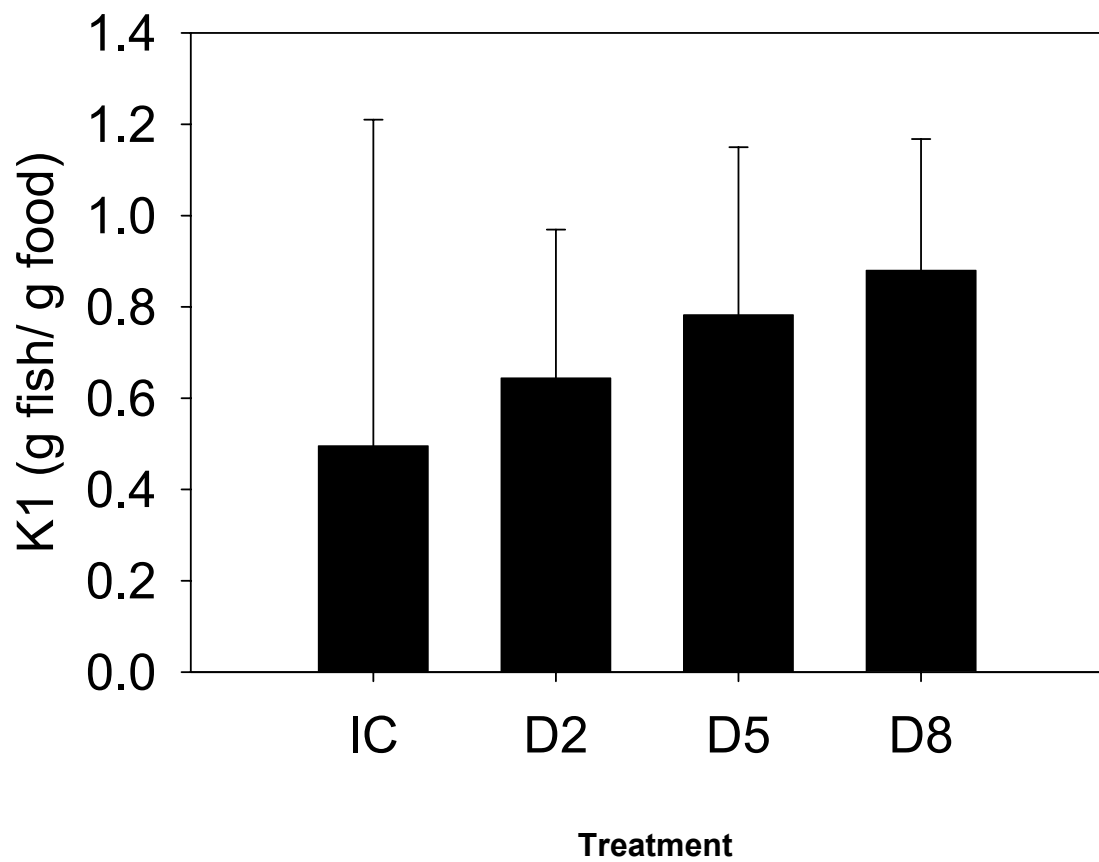


Figure 14. Mean gross growth efficiency values ( $K_1$ ) for black sea bass during PII. Vertical bars represent  $\pm 1$  standard error.

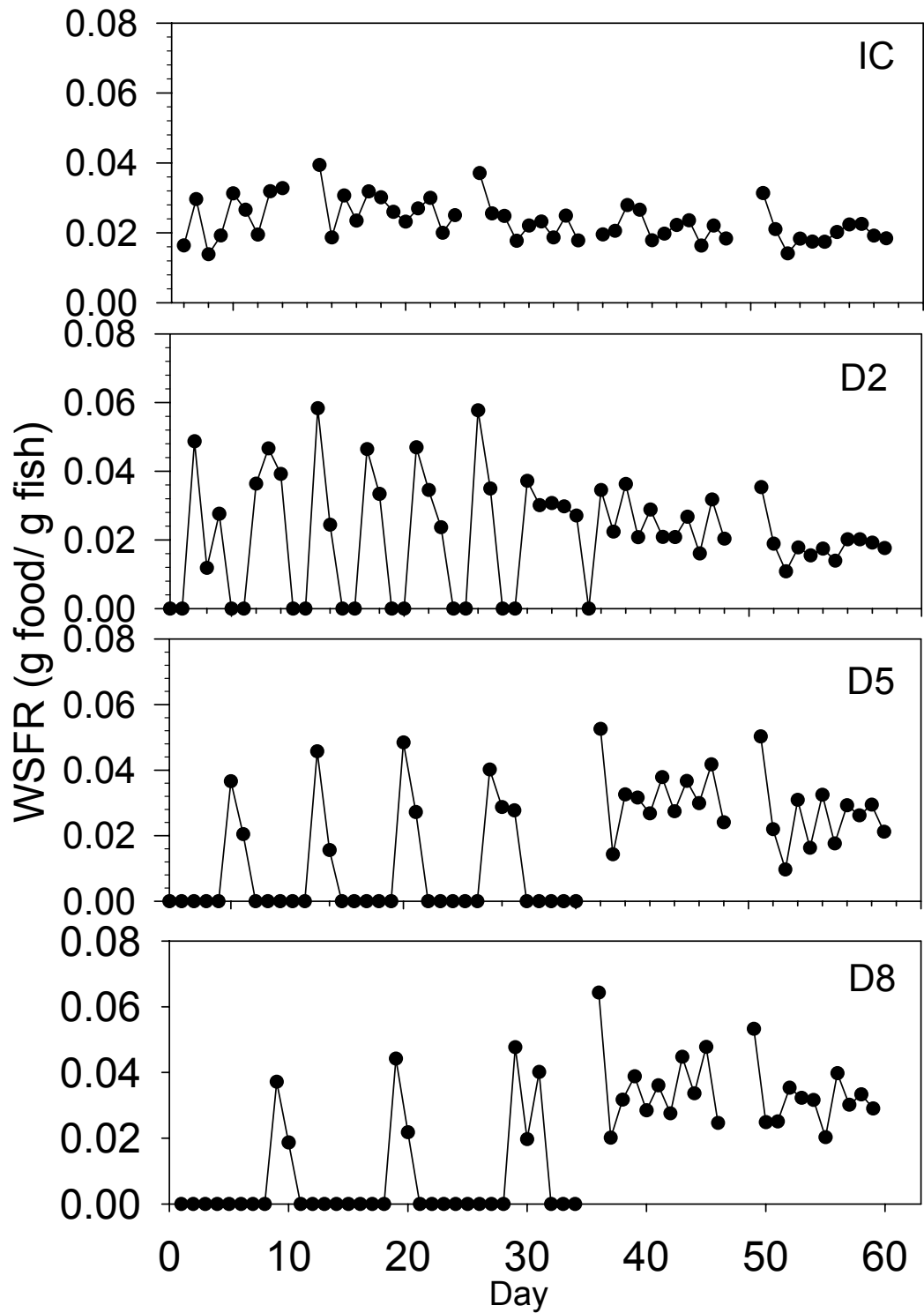


Figure 15. Daily mean WSFR values for southern flounder treatments during PI and PII. Data gaps for the IC group represent fasting periods imposed prior to weight measurements.

Southern flounder CC in PII differed significantly across treatments ( $F = 13.696$ ,  $P < 0.001$ ), with the IC consuming significantly more grams of food than any of the other treatments: IC = 11.926 ( $\pm 1.712$  g), D2 = 8.477 ( $\pm 1.213$  g), D5 = 6.045 ( $\pm 0.515$  g), and D8 = 5.819 ( $\pm 0.537$  g).

### Growth

Measurements of growth, both TL and WWT, show that the IC was able to maintain a size advantage throughout PII (Figure 16). TL showed a significant treatment effect ( $F = 11.298$ ,  $P < 0.001$ ), with the IC having significantly greater lengths than both the D5 and D8 fish. For WWT, a highly significant treatment effect resulted in PII ( $F = 9.284$ ,  $P < 0.001$ ), with the IC having significantly higher WWTs at the end of the experiment compared to the D5 and D8 treatments. There was also a significant interaction term (treatment\*interval) for PII ( $F = 3.453$ ,  $P = 0.004$ ), which indicates that the treatments did not react similarly throughout PII in terms of WWT. The IC and D2 had a greater rate of increase in WWT than the D5 and D8 treatments. Growth rates showed a significant treatment effect ( $P = 0.019$ ,  $F = 3.665$ ) during PII, with the D5 and D8 treatments showing growth rates higher than both the IC and D2 fish (Figure 17). Notably, the SGR values achieved by the D5 and D8 treatments were higher than the SGR value of the IC at any other time during the study.

### Gross Growth Efficiency ( $K_1$ )

Gross growth efficiency of SF during PII was not significantly different across treatments (Figure 18).

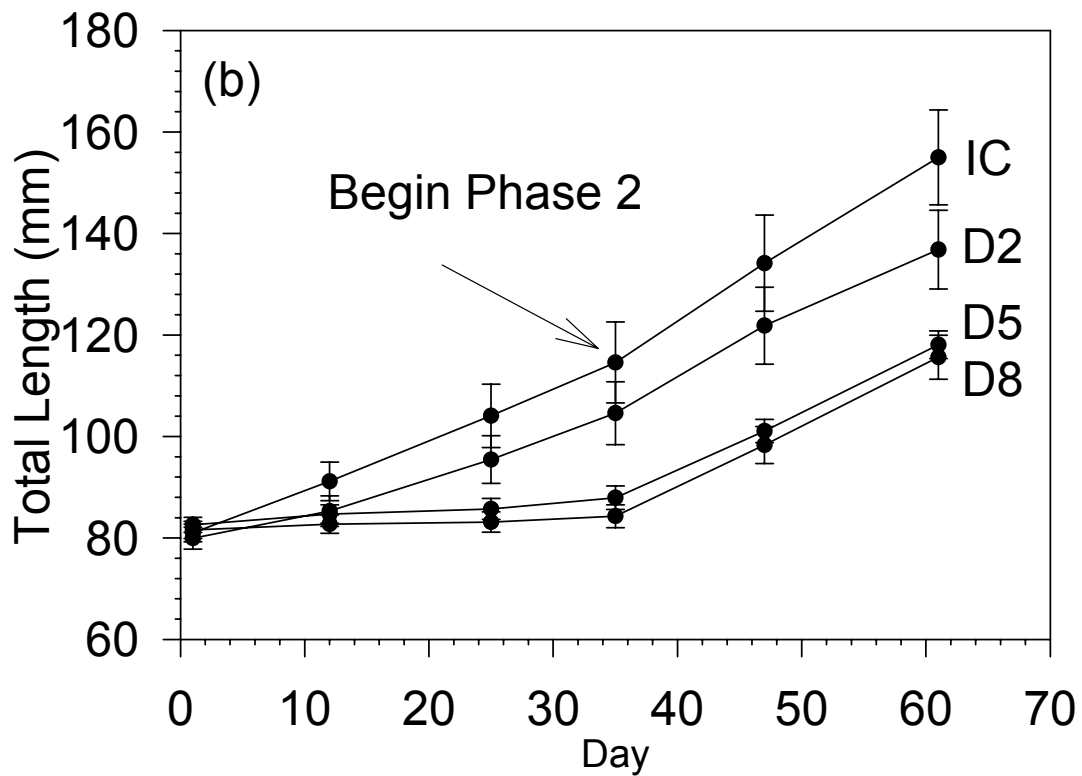
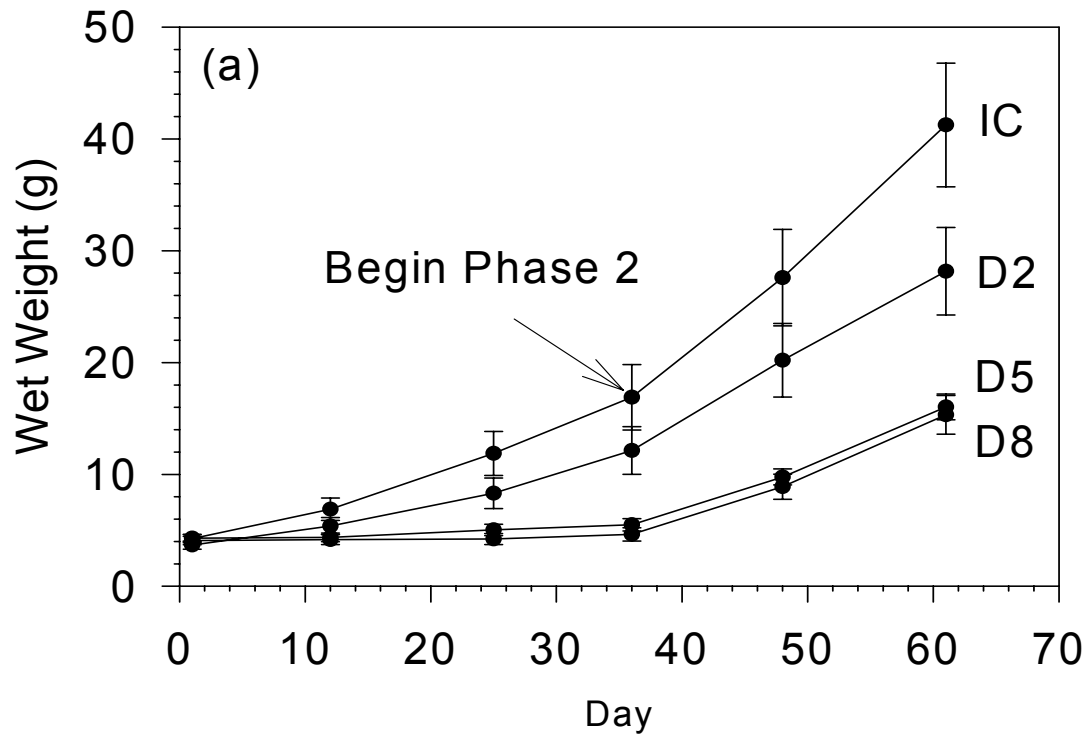


Figure 16. Mean wet weight growth curve (a) and total length growth curve (b) for southern flounder treatments during PI and PII. Vertical bars represent  $\pm 1$  standard error.



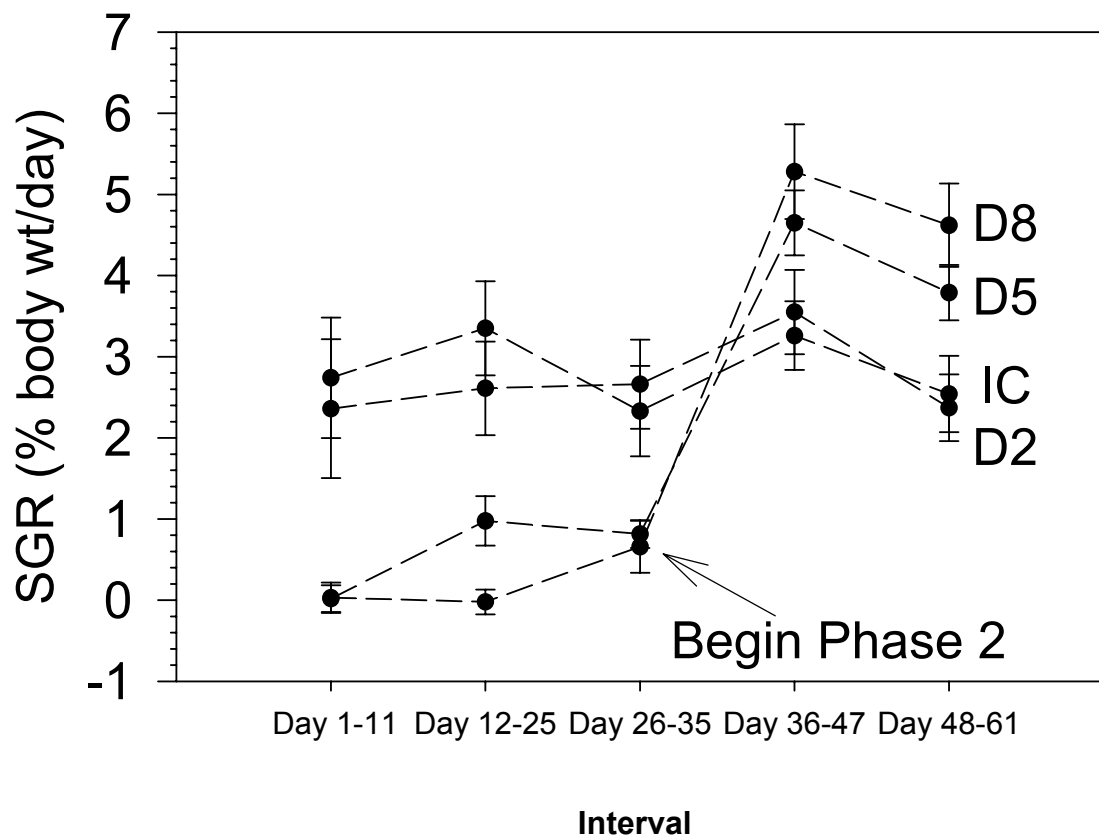


Figure 17. Mean specific growth rate values for southern flounder treatments per interval for PI and PII. Vertical bars represent  $\pm 1$  standard error.

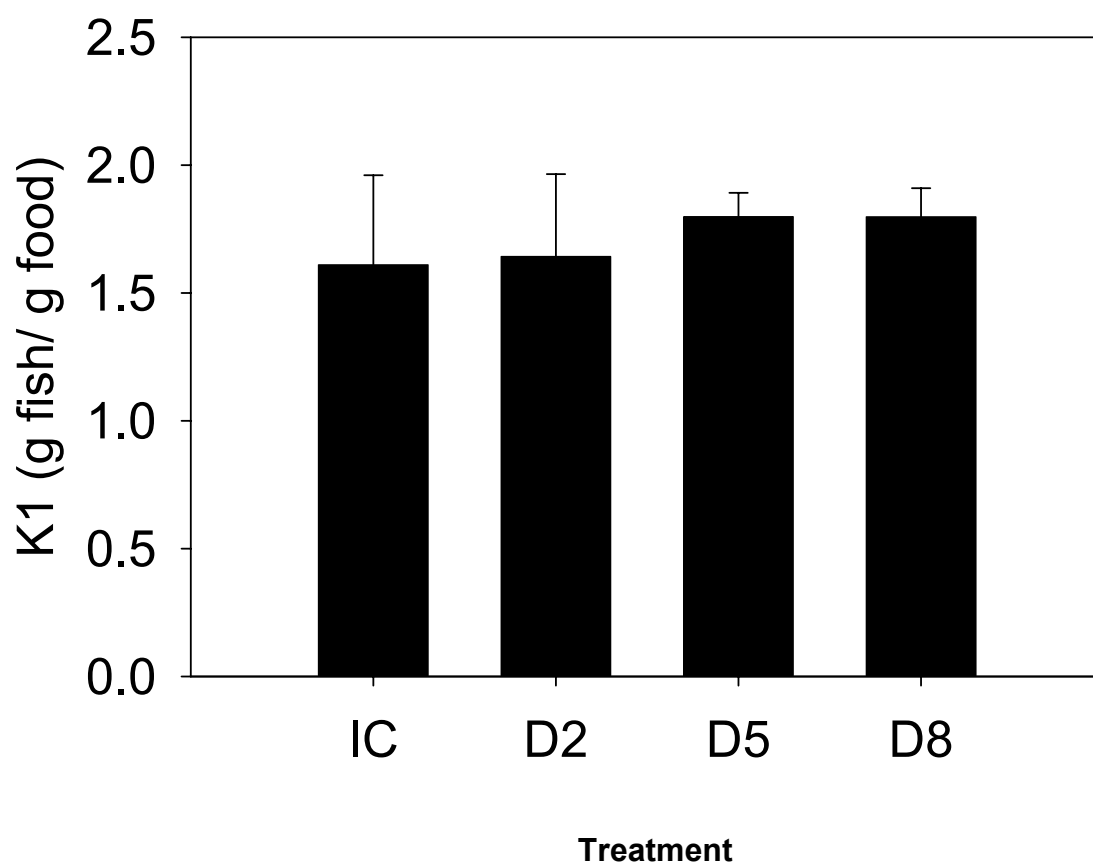


Figure 18. Mean gross growth efficiency values ( $K_1$ ) for southern flounder treatments during PII. Vertical bars represent  $\pm 1$  standard error.

## Proximal Composition

### Black Sea Bass

Proximal composition analyses showed that percent protein did not differ among treatments for BSB. Percent lipid did show a significant treatment effect ( $F = 4.512$ ,  $P = 0.012$ ) with the IC showing a higher percent lipid value than the D8 fish ( $P = 0.008$ ) (Figure 19). Thus, the trend for lipid content was:  $IC > D2 > D5 > D8$ . Percent water content of BSB varied significantly ( $F = 3.45$ ,  $P = 0.032$ ) with treatment and showed the opposite trend as the percent lipid data. The D8 fish had a higher water content than the other treatments, with the IC being significantly lower than the D8s ( $P = 0.022$ ). The hepatosomatic indices, calculated on both a wet and dry basis, showed no significant effects. Following the determination of ash free dry weight (AFDW), the analysis showed a significant treatment effect ( $F = 2.934$ ,  $P = 0.042$ ) with the IC ( $84.22\% \pm 1.88\%$ ) having a higher ( $P = 0.045$ ) ash content than the D8 fish ( $76.38\% \pm 2.59\%$ ). The visceral lipids of BSB accounted for between 44-51% of the total lipids found in these fish.

### Southern Flounder

Proximal composition analyses are shown in Figure 20. The protein content of southern flounder differed significantly across treatments ( $F = 4.9$ ,  $P = 0.009$ ). The protein content declined as the deprivation time in PI increased, thus the IC had a higher percent protein than the other treatments, with the D8s having significantly less protein than the IC ( $P = 0.005$ ). There was no significant difference in percent lipid content among the treatments. Water content also did not differ across treatments, however there was an apparent trend ( $F = 2.5$ ,  $P = 0.082$ ) for the IC to have less water than treatments

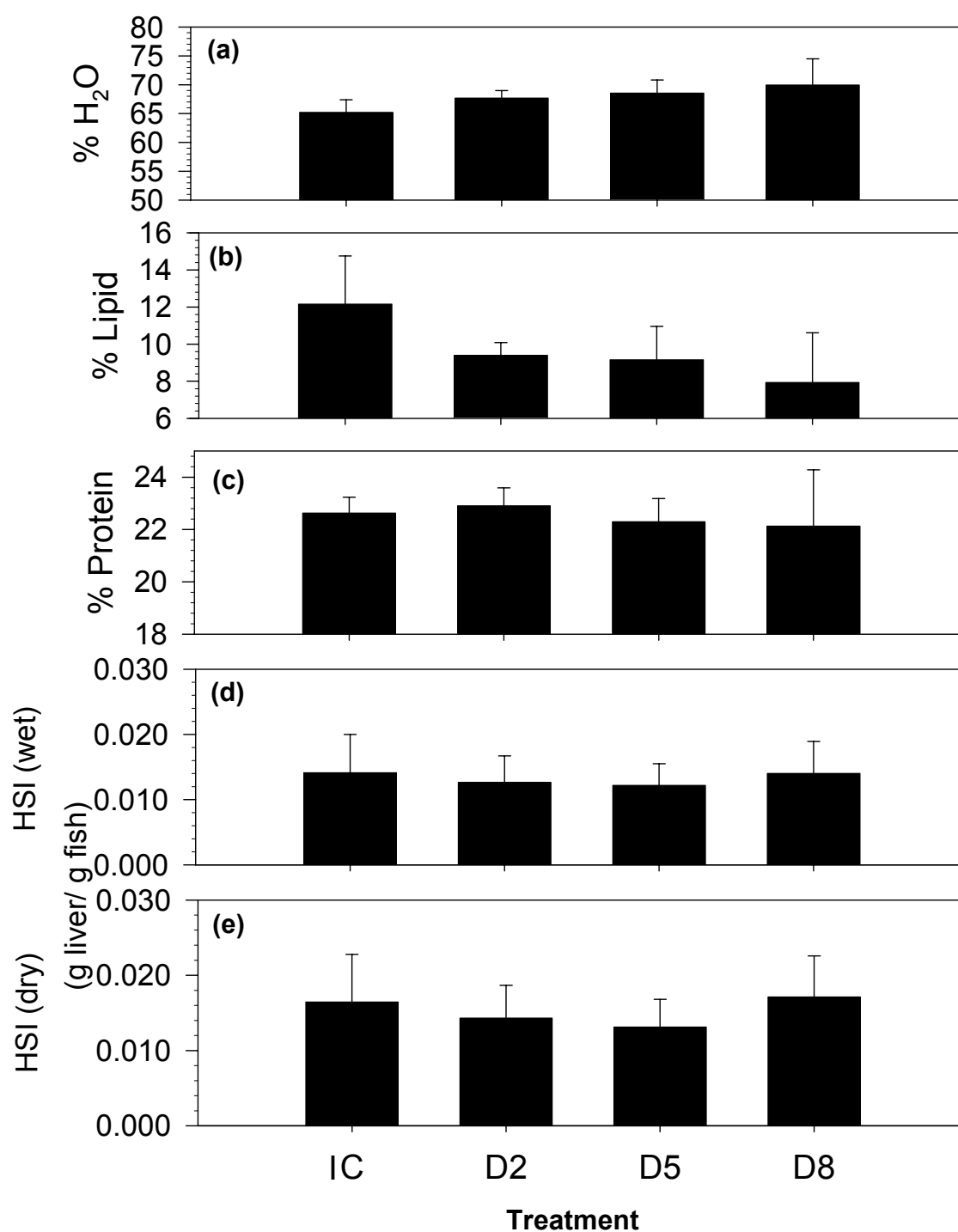


Figure 19. Proximal composition characteristics for black sea bass treatments showing percent H<sub>2</sub>O (a), percent lipid (b), percent protein (c), and hepatosomatic indices calculated on both a wet (d) and dry (e) basis. Vertical bars represent  $\pm 1$  standard error.

that were deprived during PI. Both the wet and dry hepatosomatic indices show no significant differences. AFDW did not differ significantly across treatments, and ranged from 82.54% – 84.50%. Lastly, the visceral lipids of SF accounted for less than 1% of the total lipids in this species.

#### Individual Control vs Group Control

##### Black Sea Bass

The mean WWT of the IC was significantly larger than the GC for 2 of the bi-weekly weighings (Day 46 and Day 59), however, the final WWTs were not significantly different, IC = 17.43 g and GC = 15.04 g (Figure 21). For TL, there were also 2 intervals when the lengths of the IC were significantly larger than the GC (Day 26) and (Day 46), but like the WWTs the final measurements were not significantly different, IC = 97.87 mm and GC = 93.04 mm (Figure 21).

Proximate composition analysis showed that there was no significant difference for percent water and percent lipid, but the percent protein content was significantly higher in the IC (22.63%) compared to the GC (21.48%) ( $P = 0.004$ ) (Figure 22). There were no differences found for either the wet or dry hepatosomatic indices, or for AFDW.

##### Southern Flounder

Both the WWT and TL for the SF were not significantly different between the IC and GC (Figure 23), however there was a trend for the IC to be larger (with  $P$ -values  $< 0.1$ ) at the Day 12 and Day 25 weighings. The percent water content was significantly higher in the IC (73.30%) than the GC (71.49%) ( $P = 0.002$ ) (Figure 24). No significant differences were found in the percent lipid or percent protein contents in SF. Neither the hepatosomatic indices, nor the AFDW were found to be significantly different.

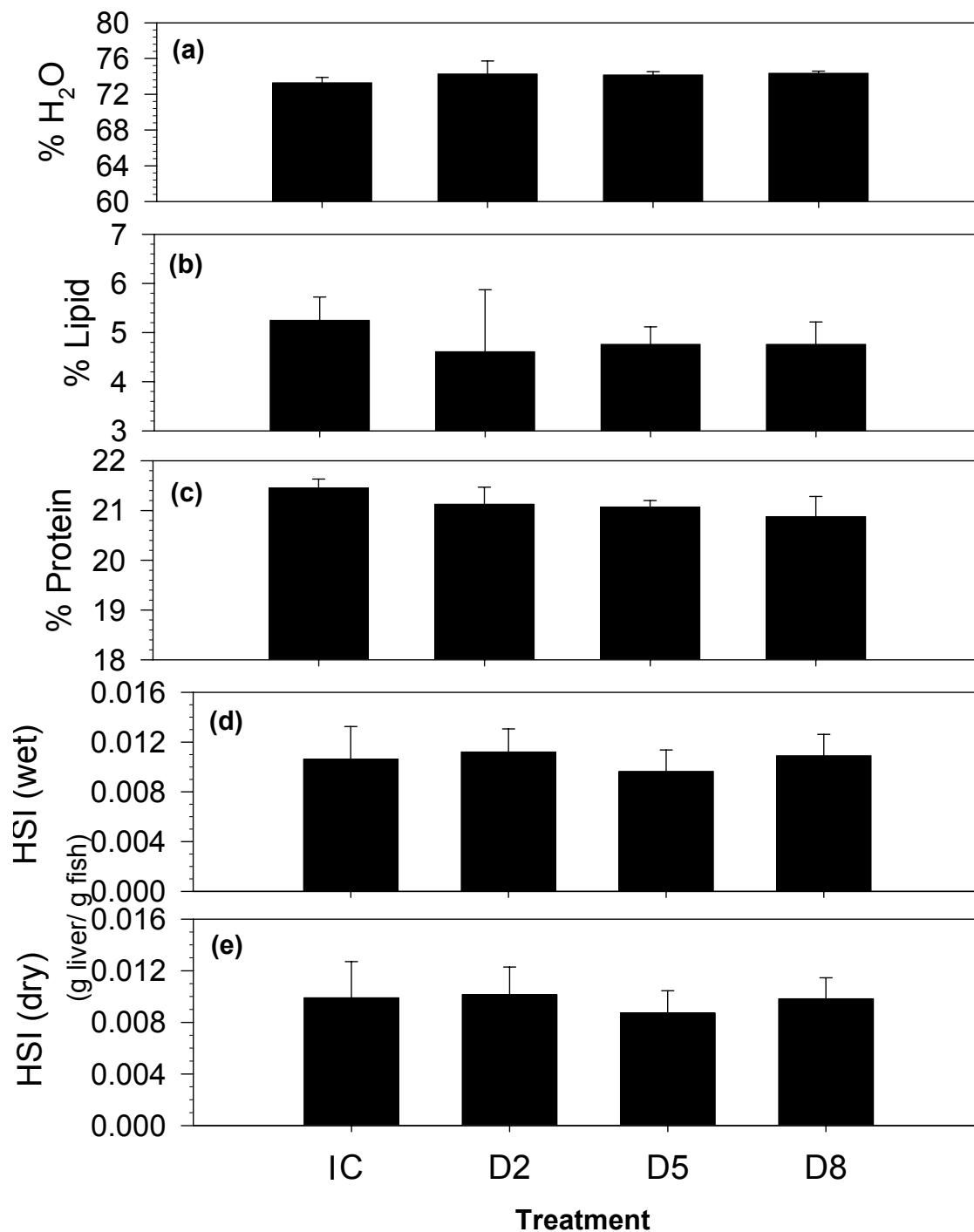


Figure 20. Proximal composition characteristics for southern flounder treatments showing percent H<sub>2</sub>O (a), percent lipid (b), percent protein (c), and hepatosomatic indices calculated on both a wet (d) and dry (e) basis. Vertical bars represent ± 1 standard error.

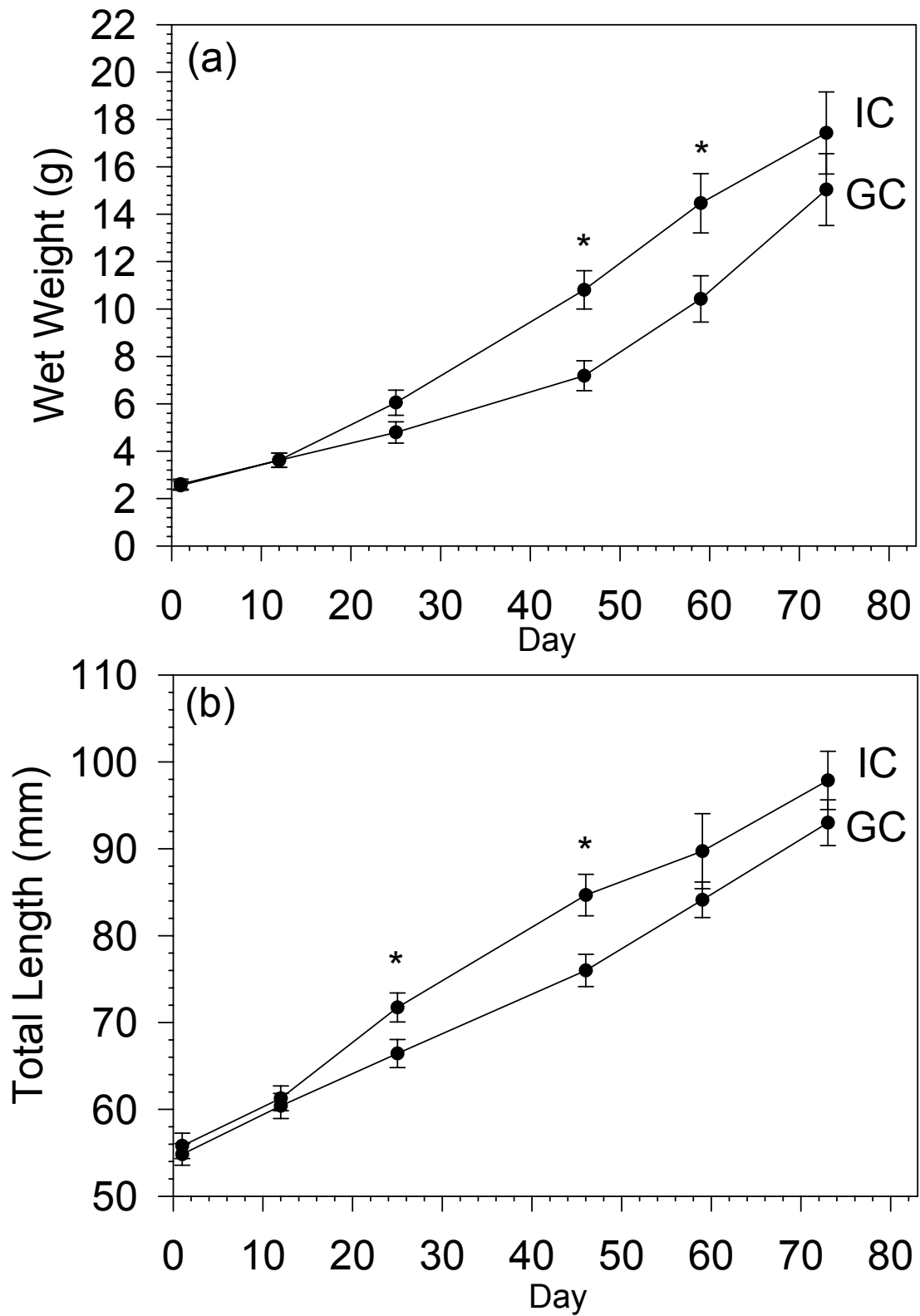


Figure 21. Mean wet weight growth curve (a) and total length growth curve (b) for black sea bass individual controls and group controls. Vertical bars represent  $\pm 1$  standard error. \* denotes  $P < 0.05$ .

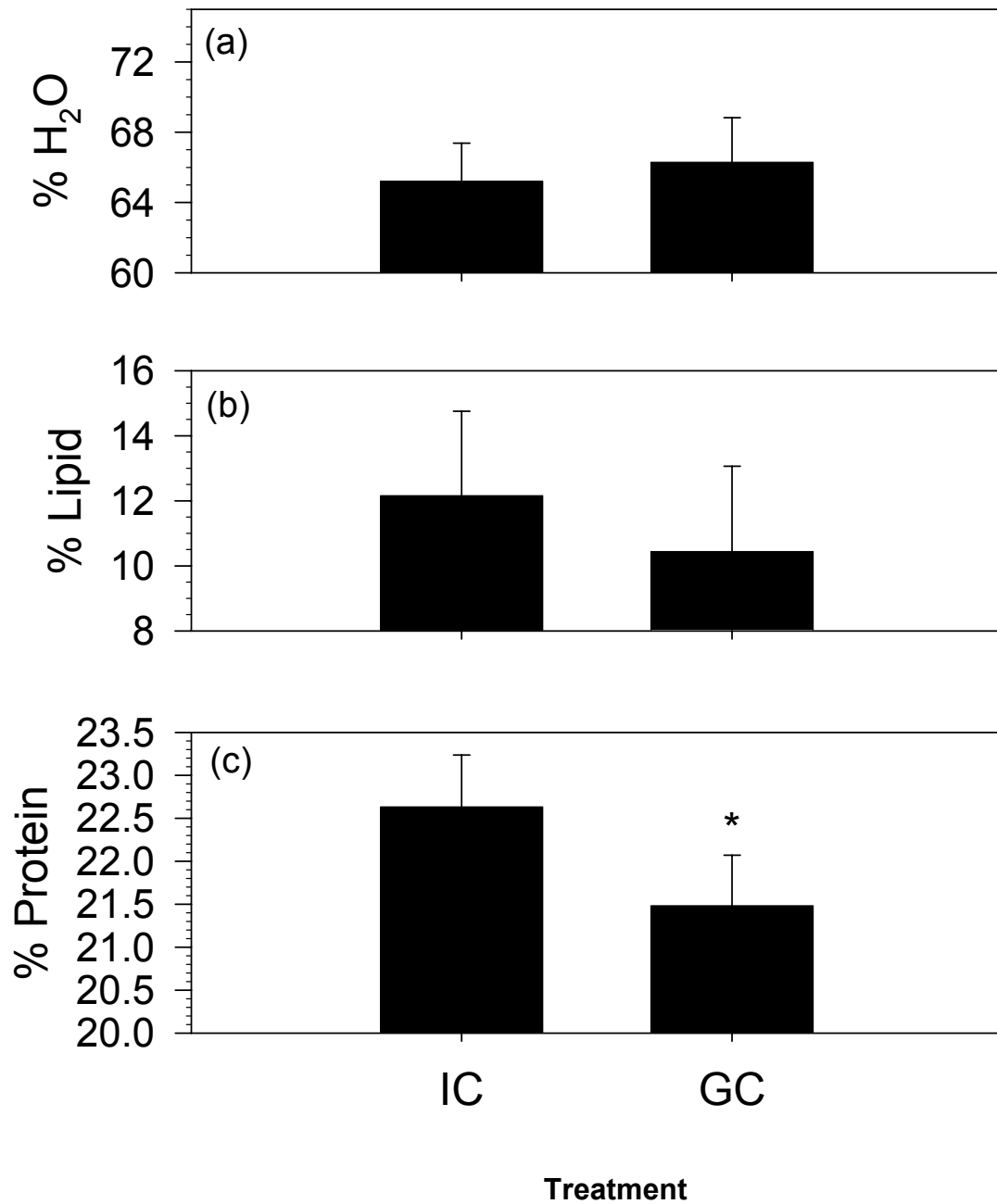


Figure 22. Proximal composition characteristics for black sea bass individual controls and group controls: percent H<sub>2</sub>O (a), percent lipid (b), and percent protein (c). Vertical bars represent  $\pm 1$  standard error. \* denotes  $P < 0.05$ .



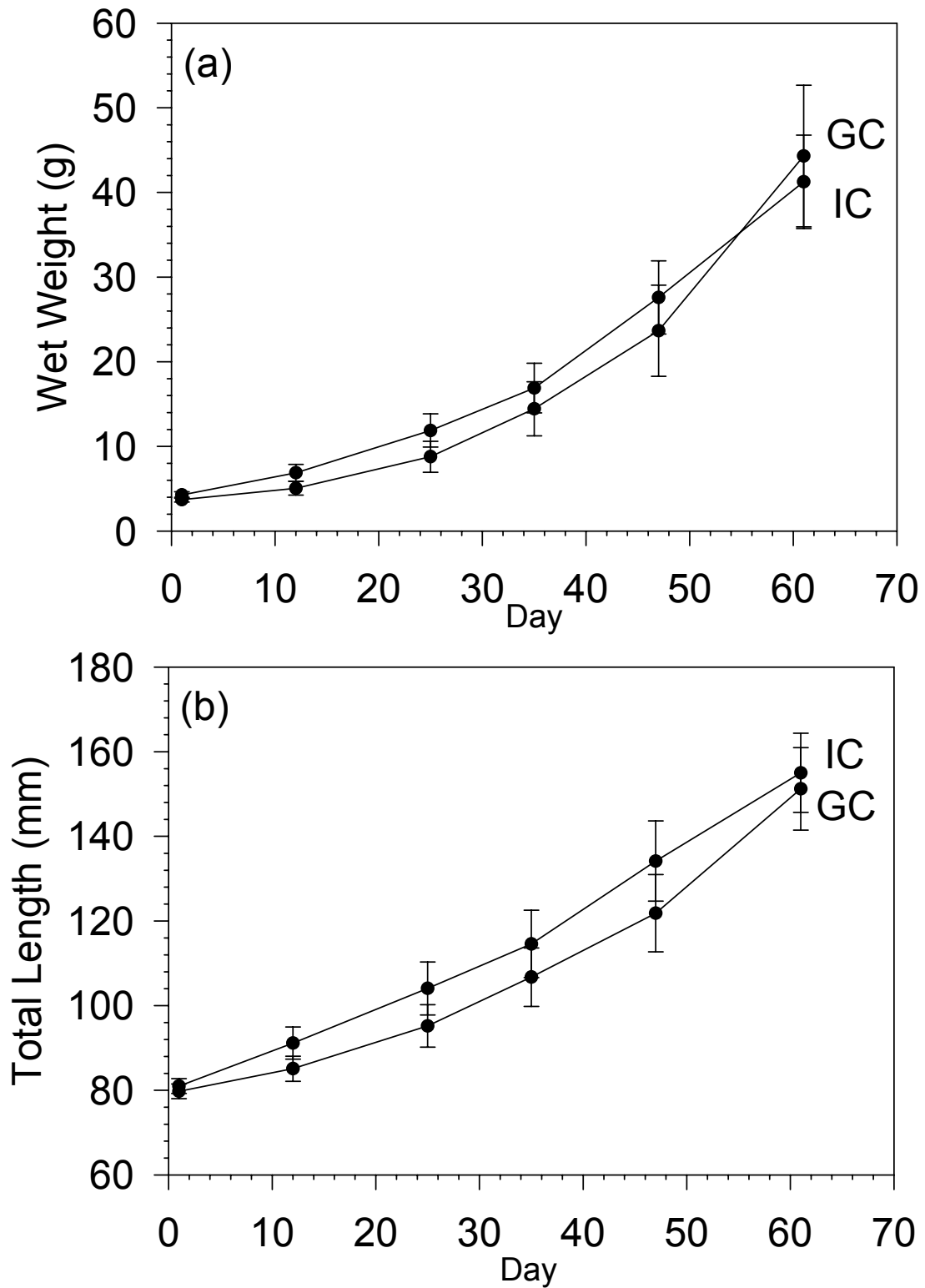


Figure 23. Mean wet weight growth curve (a) and total length growth curve (b) for southern flounder individual controls and group controls. Vertical bars represent  $\pm 1$  standard error.

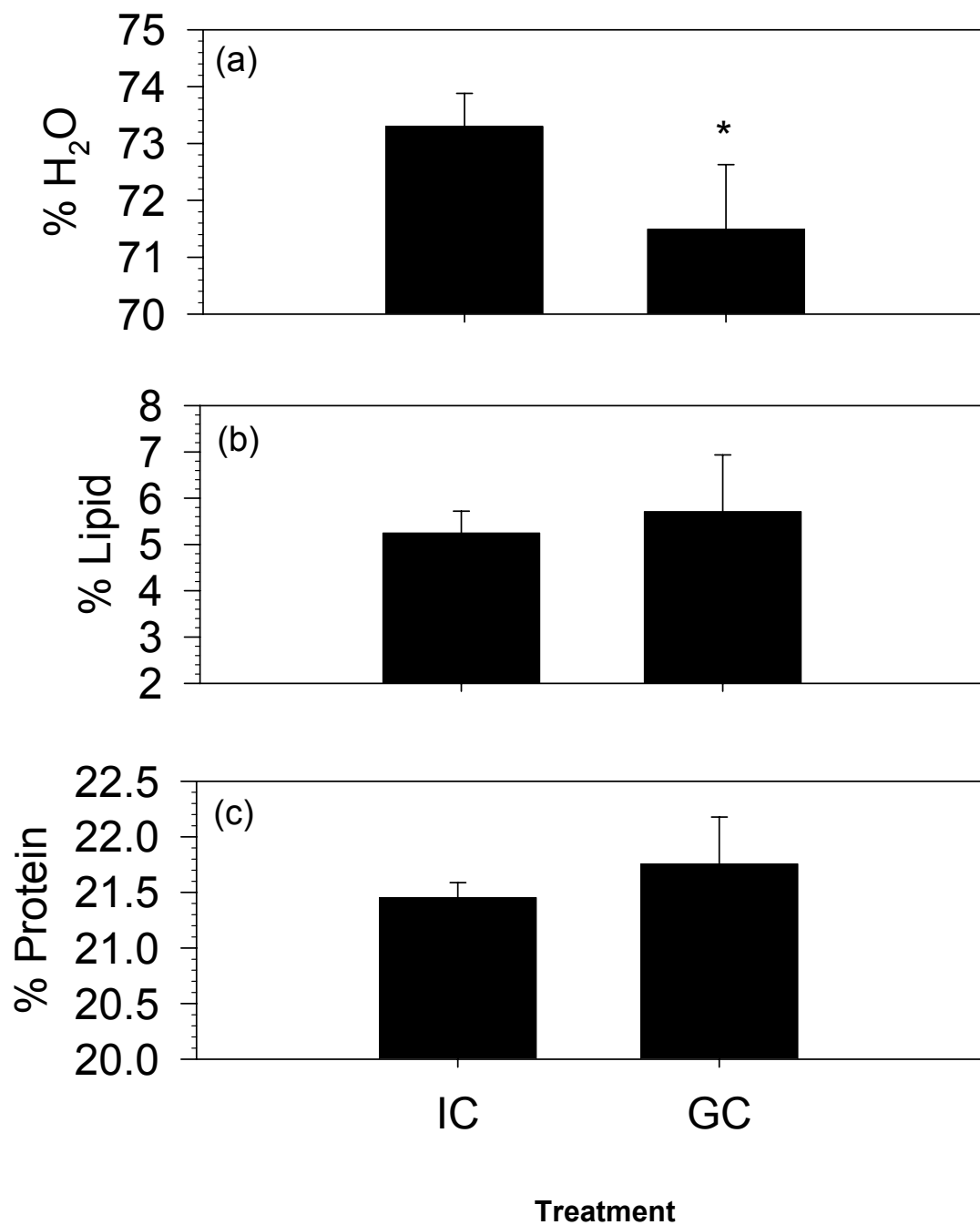


Figure 24. Proximal composition characteristics for southern flounder individual controls and group controls: percent H<sub>2</sub>O (a), percent lipid (b), and percent protein (c). Vertical bars represent  $\pm 1$  standard error. \* denotes  $P < 0.05$ .

## DISCUSSION

Previous studies of compensatory growth in fish have focused on single species, with the exception of Sogard & Olla (2002) who compared two marine finfish, and due to the different methodologies used, direct comparisons across species have not been possible. In this study however, the same methodology was used to elicit a compensatory growth response for both black sea bass and southern flounder juveniles, hence the results from these two species can be compared.

### Phase I

#### Consumption

During PI both species responded similarly to the cyclical feeding regimes. The treatments that were deprived of food showed a decreased WSFR compared to the IC, and the magnitude of the decrease was proportional to the length of time the treatment was deprived. Comparing the WSFR values between the 2 species shows that the BSB IC had a 55% higher WSFR than the SF IC, suggesting that the BSB have a higher intrinsic feeding rate compared to SF. The BSB treatment groups also ate at a higher rate than the SF treatments, by 23% in the D2s, 99% in the D5s, and 82% in the D8s. Initial size differences were not responsible for this difference in WSFR. Based on the results of an ANCOVA, the species are significantly different ( $F = 9.093$ ,  $P = 0.006$ ) after adjusting for differences in initial size.

The most notable feeding response during PI was the short duration of hyperphagia following periods of deprivation. In terms of WSFR, both species showed signs of hyperphagia in treatment groups by significantly exceeding the WSFR of the IC groups. In most cases though, the SF did not show any hyperphagic response according

to the parameters set out in the methods which were used in this study. However, during the times when a hyperphagic response was detected, it was short-lived and upon re-feeding the hyperphagia lasted no more than one day regardless of treatment. This result was surprising because the hyperphagic response of fish in a previous study (Hayward *et al.*, 1997) to similar deprivation treatments lasted much longer and was proportional to the amount of time the fish were deprived.

The limited duration of hyperphagia, and lack of full compensation observed in this study can be viewed in the context of competing hypotheses regarding the fitness consequences of rapid growth rate. The first hypothesis deals with constraints on digestion rate. The fish may not be capable of increasing their digestion rate sufficiently to process the increased food intake which occurred upon re-feeding, thus the fish would have been physically incapable of maintaining such high feeding rates. The second hypothesis is that submaximal growth might be adaptive and therefore preferred; the fish chose not to continue feeding at such high rates due to certain disadvantages caused by high ingestion rates such as decreased swimming capability and increased vulnerability to predation (Billerbeck *et al.*, 2001; Lankford *et al.*, 2001). The latter explanation supports the theory that juvenile fish growth rates are strategic and may be optimized below a maximal rate in order to increase their fitness (Schultz *et al.*, 2002).

Cumulative consumption calculated as g food/fish/day shows that the SF IC ate 10% more food than the BSB IC during PI. Again, the size difference of the fish used for the study needs to be considered since the BSB were smaller than the SF. Notably, during PI the BSB D5 and D8 groups ate 73% and 50% more than their respective SF

counterparts. This finding demonstrates that BSB are capable of increasing food intake more than SF during periodic deprivation conditions.

### Growth

During PI, the most deprived treatment groups (D8) of both species experienced considerable hardship due to the deprivation imposed by the cyclical feeding regimes. At the end of PI, for example, the D8 treatments weighed 56% and 42% of the IC for the BSB and SF, respectively, and the total lengths of the D8 fish were 87% (BSB) and 85% (SF) of their respective IC. In general, the SGR values of the BSB were higher than the SF during PI, with the BSB IC having growth rates that were 8% higher than the IC for the SF. However, based on an ANCOVA, there is not a species effect ( $F = 3.462$ ,  $P = 0.0749$ ) and the difference in growth rate can be explained by the difference in initial weight ( $F = 6.661$ ,  $P = 0.015$ ). Both species showed decreased SGR values as the deprivation time of the treatment increased, but for the SF the decrease was much more pronounced.

### Gross Growth Efficiency ( $K_1$ )

A comparison of the  $K_1$  values between the species shows that the SF have intrinsically higher growth efficiencies than the BSB, with the SF IC showing a 52% higher  $K_1$  than the BSB IC. However, during PI the BSB D8, SF D5 and SFD8 treatments experienced negative  $K_1$  values (Individuals with negative  $K_1$  values: BSB D8  $n = 2$ , SF D5  $n = 3$ , and SF D8  $n = 5$ ) indicating that those treatments actually experienced weight loss. This would suggest that SF are less efficient at converting food into growth than BSB during times when food resources are low.

## Phase II

### Consumption

After the cyclical feeding regimes were ceased and the fish were returned to unlimited rations some interesting patterns emerged. The treatments which were most deprived sustained a higher WSFR for the entire duration of PII, and the BSB had consistently higher WSFR values than the SF in each treatment. Interestingly, for both species the D5 and/or D8 treatments showed significantly higher WSFRs compared to their respective controls. For BSB, both D5 and D8 treatments were 53% higher than IC ( $P < 0.05$ ), and for SF the D5 ( $P < 0.05$ ) and D8 ( $P < 0.1$ ) fish showed WSFR values which were 38% and 46%, higher than their IC, respectively. In terms of feeding rate, there appeared to be a threshold between the D2 and D5 treatments for both BSB and SF, since the D2 treatments of each species showed only small increases in WSFR during PII. For BSB there was only a 7% increase in feeding for the D2s, and for SF the D2 fish increased their feeding rate by a mere 5% -- both of which were not significantly different than the feeding rates for their respective IC.

Cumulative consumption during PII of the experiment showed increases in the amount of food eaten per fish per day compared to PI for all treatments and controls, with the SF generally having higher overall consumption. Again this is may be due to the larger size of the SF juveniles used for this study. Notably, the BSB D5 fish had a 20% higher consumption in PII than the BSB D2 treatment, and an 11% higher consumption compared to their SF counterparts. This marked increase in consumption indicates that the BSB D5 fish were actively using increased feeding to compensate their growth to an extent that exceeded the other treatment groups.

## Growth

Full compensation of body size was not achieved by either of the species that were used in this experiment. All treatment groups were smaller in terms of both WWT and TL than the IC fish at the end of PII. However, there is evidence of partial compensation in certain treatments. For example, the BSB D5 treatment was able to approach the BSB D2 treatment in size, 85% in WWT and 97% in TL, despite experiencing deprivation which lasted 2.5 X that of the D2 fish. The increased consumption of the BSB D5 fish in PII, mentioned previously, was done as an attempt to catch up in growth.

Growth rates in PII reflect the WSFR, in that the most deprived treatments show the highest SGR values. Overall, the SF showed higher growth rates compared to the BSB in PII with the SF IC having a 78% higher SGR value than the BSB IC. In PII, the SF were certainly able to accelerate their growth rates above the levels of the BSB, and the only BSB treatment able to produce a SGR value in the same range as the SF was the BSB D8s. Also, similar to the results from WSFR, observations that a threshold exists between the D2 and D5 treatments for both species were seen with respect to growth rate. D5 and D8 treatments in BSB were respectively 64% and 86% higher than BSB IC, and for SF the D5s were 46% and D8s were 71% higher than controls. Meanwhile the D2 treatments were only 1% higher in the BSB, and 2% higher in the SF during PII. Due to the minor differences between the IC and D2 treatments with respect to WSFR and SGR values, these findings suggest that it may be worthwhile to perform a cost/benefit analysis to determine whether there would be advantages to raising either species under a D2 feeding regime. Although the highest growth rates were found in the D8 treatments, the

severity of the deprivation during PI appeared too great for them to fully compensate by the end of PII. Perhaps if the second phase were continued for a longer period of time, the D5 and D8 treatments may prove to be able to fully compensate, however this investigation suggests that the implementation of a D5 or D8 feeding regime for commercial production would not be profitable.

#### Gross Growth Efficiency ( $K_1$ )

Again, like the WSFR and growth rate results, the most deprived treatments from PI had the highest  $K_1$  values in PII, indicating that the cyclical feeding imposed during PI produced increased growth efficiencies during PII (Ali *et al.*, 2003). SF were able to achieve  $K_1$  values which far exceeded those of the BSB. The SF IC had growth efficiencies 325% higher than BSB controls. This finding further supports that the SF have intrinsically higher growth efficiencies than BSB. Evidence for a certain threshold exists for the SF with respect to  $K_1$ , with D5 and D8 treatments exhibiting 12% higher growth efficiencies and the D2s which showed a mere 2% increase over the controls. The BSB showed no sign of a similar threshold with a steady trend for increase in  $K_1$  from D2 (at 30% above IC) to D8 (at 78% above IC).

#### Proximal Composition

The proximal composition analysis has shown that these 2 species have different body compositions with respect to non-polar lipid, protein, and water content. Protein content remained the most similar of these components between BSB and SF and accounted for approximately 21-22% of the WWT of these fish. However, both water content and non-polar lipid content were different between the species. BSB contained a higher percent of non-polar lipid than the SF, and ranged from 8% to 12% of their WWT



across the different treatments, whereas the percent lipid content of SF was lower and less variable across the treatments (4.6-5.2%). This pattern may help to explain some of the disparity in  $K_1$  because lipids, although more energy rich, are less dense than protein and would not contribute as much as protein to an increase in WWT. The water content of BSB was lower, ranging from 65-70%, compared to SF water content which accounted for 71-74% of their WWT. Interestingly, these fish appear to have different storage patterns for lipids. Visceral lipids accounted for 44-51% of the total lipids in BSB, whereas SF stored less than 1% of their total lipids in their viscera. In general, the BSB were fattier than the SF, and this result may indicate that the BSB have a lipid buffer which they use for energy during times when food availability is low. This finding may explain why the protein content of BSB were not significantly different across treatments, while SF D8s showed significantly less protein than the SF IC. Interestingly, the BSB D5 which showed compensation relative to BSB D2 did not show any notable changes in their proximal composition.

When there were significant differences within a certain body component, the disparity among the treatment groups was different across species. For BSB, percent lipid for the D8s was significantly lower than for the IC which indicates that the D8 treatment either tapped into their stored lipid buffer, or simply could not store as much as the IC. Since proximal composition analyses were done at the end of PII, these fish had the entirety of PII to restore lipids, so the depletion that was observed may not represent accurately the true severity of lipid depletion which occurred after PI. Also in BSB, the D8 fish showed a significant increase in percent water content which may suggest that they artificially maintained mass by incorporating more water into their tissues (Ali *et al.*,

2003). Similar findings have been noted for brook trout (Cox, pers. comm.), and Atlantic cod (Black & Love, 1986).

The SF did not have any type of observable lipid buffer like the BSB, consequently they may have needed to readjust their allocation approach during PI when food was severely restricted. SF D8s may not have been able to maintain protein production at the same level as controls, and hence percent protein content of the D8s was significantly lower than the IC. Although not significant, the SF D8s showed a higher mean water content ( $P < 0.1$ ) similar to the BSB D8s. Since this was observed in both species, it may be possible that this is a way for fish which are severely deprived of food to artificially maintain their mass. However, this increase in water content would be undesirable for aquaculture production since it would cook off when the fish was prepared.

#### Individual Controls versus Group Controls

The purpose of comparing the individual controls to the group controls was to examine whether there was an effect of group holding on parameters important to aquaculture production. Neither of these species were affected in terms of growth in WWT and TL by being held in groups. However, proximal composition analysis showed that for BSB, individually held control fish had a significantly higher protein content (22.6%) than the fish in the group controls (21.5%) suggesting the IC fish were in better condition. Also, for SF the proximal composition analysis indicated that the water content of controls (73.3%) held individually was significantly higher than those held in groups (71.5%). Therefore, group holding does have significant effects on body composition on fish but does not significantly affect growth rate. The minor differences

found in protein content of BSB and water content of SF would not be sufficient cause to hold fish individually for aquaculture production due to the higher costs of doing so.

#### Ecological/Life-History Differences

The findings of this investigation may indicate that these two species have different approaches toward compensating growth. The BSB appear to utilize an increased feeding rate, as evidenced by higher WSFR values during the re-feed periods of PI and consistently higher WSFRs for the duration of PII, as a means to grow after times when food is scarce. The SF also rely on increasing their food intake, but in addition they appear to have higher growth efficiencies, and combined they utilize these two responses to maintain growth during periods that follow times of low food availability. Both strategies can be equally effective at compensating growth and the difference in the ways that these species utilize feeding rate and growth efficiency may be indicative of differences in their life-histories. The most severely deprived treatments showed the greatest response to the cyclical feeding regimes in terms of WSFR, growth rate, and  $K_1$ . Similar findings have been observed in previous studies (Jobling, 2001; Wieser *et al.*, 1992).

As an estuarine-dependent species, the southern flounder are prone to experience more seasonality than black sea bass which are a reef-associated species. Both temperature and prey abundance in estuaries may fluctuate throughout the course of a year to a greater extent than on an offshore reef. Patchiness is also expected to be greater in an estuary than a reef since the area an estuary comprises is generally much larger than a reef. Thus food resources may be diffuse in estuaries and more concentrated on reefs.

The different strategies of these species are probably a reflection on their different ecologies. Black sea bass are reef-associated as adults, and although reefs are highly productive, the literature has not indicated that they consume prey that is necessarily found only on reefs, thus they use reef habitat more as a shelter than a food resource (Steimle & Figley, 1996). However, the productivity that reefs support may cause the surrounding sea bottom to become enriched (Steimle & Figley, 1996) and may generate a food base in the sandy bottom areas adjacent to reef structure. Since BSB in North Carolina have been shown to make substantial use of prey found on sandy bottom (Lindquist *et al.*, 1994), the reef habitat they utilize for shelter may also be responsible for providing them with a continual food supply. Thus, since BSB may experience less frequent disruptions in food availability and less seasonal temperature variations, they should not have had the need to evolve a strong compensatory response.

For SF who prefer to live in areas of soft, muddy bottom (Burke *et al.*, 1991), far up towards where the river empties into the estuary, a higher seasonality gradient and increased patchiness may occur, which could have caused SF to evolve the need to compensate growth, and develop a higher intrinsic gross growth efficiency to do so. This way, on occasions when food is available for them to eat, they should get more growth from less food. So, in addition to increasing food intake, SF also dramatically increased their growth efficiencies, and thus showed a stronger ability to compensate their growth.

In terms of the larger ecological scheme, there may be a continuum in compensatory ability which depends upon the life-history of individual species. Those species which inhabit highly seasonal temperate latitudes, such as the hybrid sunfish used in Hayward *et al.* (1997), may have evolved a higher capacity to compensate growth than

species that live in more stable tropical latitudes (Wang *et al.*, 2000). Thus, one could expect an estuarine-dependent species like SF to have a higher capacity for compensation than a reef-associated species such as BSB. Currently, there is a lack of information for strictly marine species, and more studies are needed which use ecologically diverse species of fish to better understand the evolutionary significance of compensatory growth (Ali *et al.*, 2003).

#### Evaluation of Technique Used

This investigation was modeled after Hayward *et al.* (1997) in which hybrid sunfish were successfully manipulated into growing at twice the rate of controls. The same technique used in that study to elicit compensatory growth on a species that is heavily influenced by the seasonality of temperate, inland ponds and lakes was employed on two promising aquaculture species in the present study. Over-compensation observed by Hayward *et al.* (1997) was not observed in this study; neither BSB nor SF fully compensated their growth in terms of size. In fact, the results from Hayward *et al.* (1997) have not been duplicated by any study thus far; including a study done by Hayward *et al.* (2000) which used the same species and similar feeding protocols. Implicit to the use of this technique is to gauge hyperphagia in order to know when the next deprivation period should be imposed. Hyperphagia is measured on a weight specific basis, and in an experiment such as this it would be ideal to know the exact weights of the fish on a daily basis, however this would be impractical because the disturbance to the fish would be so great that it would undoubtedly interrupt natural feeding behavior. Bi-weekly weighings were used, however as the time increased since the last weighing WSFR was continually over-estimated (Figure 25). Thus, a better approach to elicit a compensatory growth

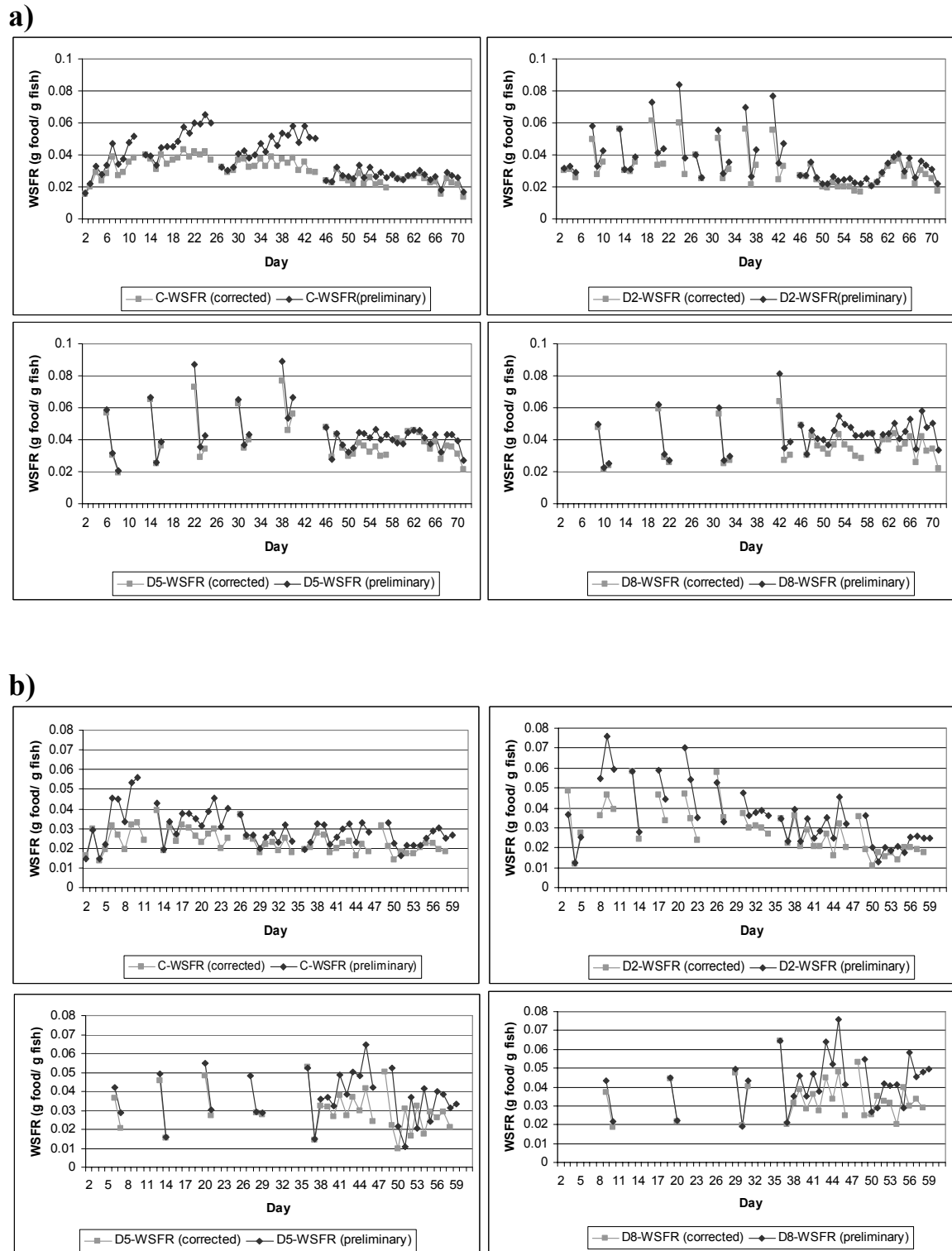


Figure 25. Mean weight specific feeding rate (WSFR) vs. day for each treatment of black sea bass (a) and southern flounder (b) showing the preliminary and corrected WSFR values.

response may be to set both the number of deprivation days and the number of re-feeding days rather than calculate the duration of hyperphagia.

Additionally, it is possible that the refeeding periods in PI were not long enough to allow for the necessary physiological adjustments needed to up-regulate digestion during times of increased feed intake. Evidence exists for structural changes in the gut which increase its capacity (Carter *et al.*, 2001), however, these are long term changes which could not have occurred during the re-feed periods of PI, hence intake rates were limited by the maximal rate at which food could be digested (Ali *et al.*, 2003). Thus, the two day requirement for significantly higher WSFR values used to test for the presence of hyperphagia in PI may not have been long enough for these fish to enter into a state where increased feed consumption would be possible. However, in PII the deprived treatments were able to maintain higher WSFRs than the IC fish for the duration of PII, perhaps because they were allowed enough time to re-adjust their digestion rates and/or gut capacity to handle increased consumption.